TARGETED DRUG DELIVERY OF CAPECITABINE MICROSPHERES FOR COLORECTAL CANCER

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Peelamedu, Coimbatore – 641004

Certificate

This is to certify that the dissertation entitled TARGETED DRUG DELIVERY OF CAPECITABINE MICROSPHERES FOR COLORECTAL CANCER was carried out by JOHN.R, in the Department of Pharmaceutics, PSG College of Pharmacy, PSG Institute of Medical Sciences & Research, Peelamedu, Coimbatore, which is affiliated to The Tamilnadu Dr. M.G.R. Medical University, Chennai, under the direct supervision and guidance of Dr. C. Vijaya Raghavan M.Pharm., Ph.D. Vice Principal and Head, Department of Pharmaceutics, PSG College of Pharmacy, PSG IMS & R, Coimbatore.

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SCOPE AND PLAN OF WORK

Colorectal carcinoma is the second leading cancer killer and third most common cancer through out the world .The incidence of colorectal cancer is second for men next to lung cancer and second in women next to breast cancer. Surgery, radiation therapy and chemotherapy are the three type of treatment commonly used. The main problem in Colorectal cancer is that 30-50% of patients have advanced disease when they are diagnosed and occurs most frequently after the age of 50, approximately 7% are seen in patients less than 40 years of age.

Capecitabine is a new drug for orally administered drug is widely used for the treatment of colorectal cancer. It is readily absorbed from gastrointestinal tract. It is a fluoro pyrimidine which is converted into 5-fluoro uracil (5-FU) after entering the tumour cells. It is unstable in acidic condition of the stomach. Less than 50% is bonded to plasma protein, It has a peak plasma concentration of 1.5 hours. It is metabolized in the liver by 60 K Da Carboxylestesterase. By converting into controlled, site specific release dosage form first pass metabolism can be minimized, bioavailability can be increased, deactivation in gastric pH can be avoided, reduce the dose frequency and minimize side effects when compared with

the conventional treatment.

The approach to achieve colonic delivery of drug is by following methods.

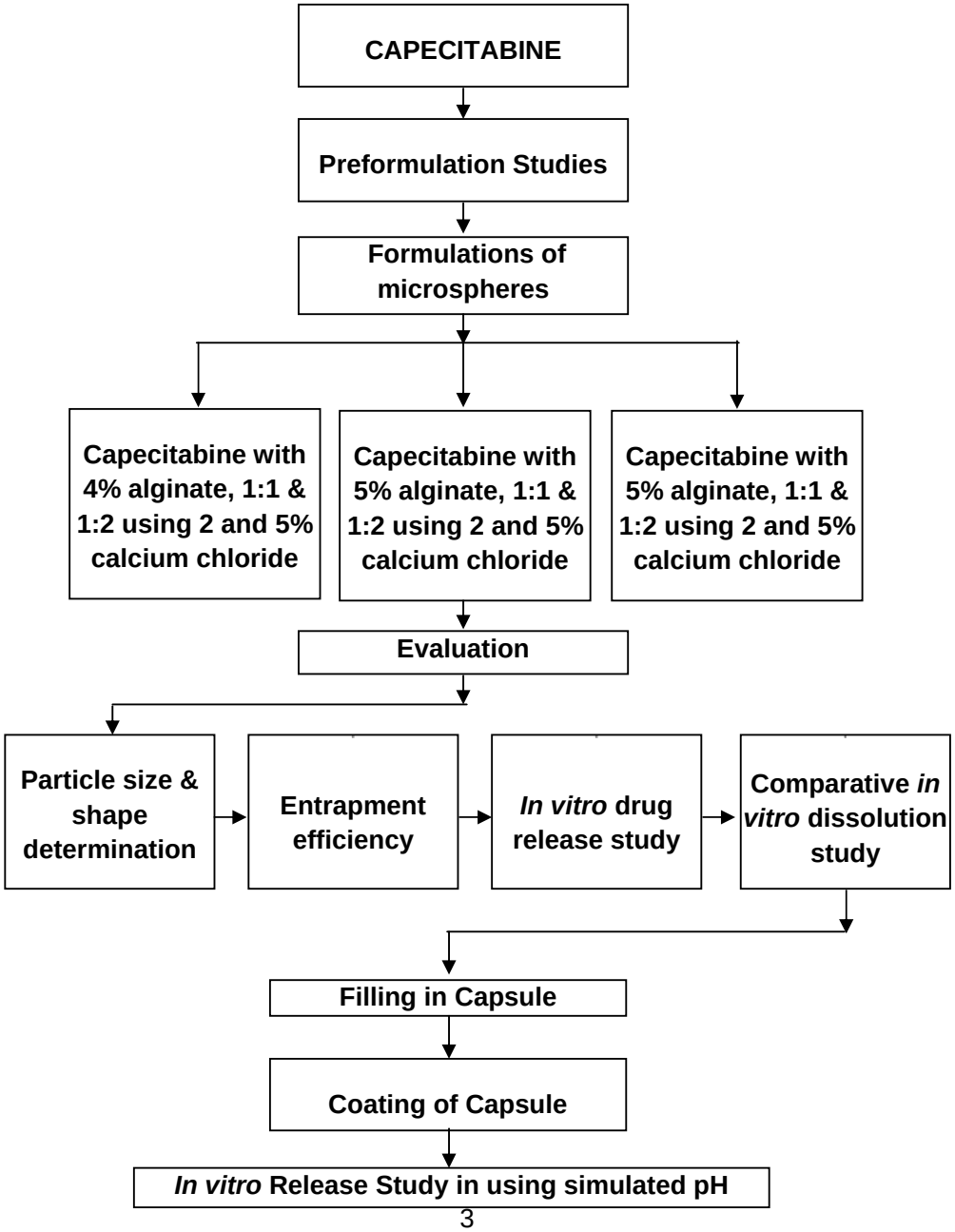
1. Coating with pH dependent polymer
2. Timed release dosage forms
3. Delivery system based on the metabolic activity of colonic bacteria
4. Osmotic controlled drug delivery

The pH dependent systems exploit the generally accepted view that pH of the human gastrointestinal tract increases progressively from the stomach (pH1-3), small intestine (6.5-7) to the colon (7-8). Most commonly used pH dependent coating polymers are methacrylic acid copolymers – Eudragit S100 which dissolve at pH 7.5. The use of Eudragit S100 prevented drug release in the upper gastrointestinal tract and permitted selective drug delivery in the colon.

The present study involves formulation, characterization and *in vitro* release study of capecitabine containing sodium alginate microspheres using Eudragit S100 coated capsules for the treatment of colorectal cancer

Chapter 1
Scope & Plan of Work

SCHEME OF STUDY



INTRODUCTION

Oral administration has been the traditionally preferred route of administration for most therapeutic agents and is in general the first avenue investigated in the discovery and development of new drug candidates and formulation.

Owing to patient acceptance convenience of administration cost effective manufacturing that does not required sterile processing and generally long product shelf life that is often dictated more by the stability of active drug itself rather than the formulation components. A continued emphasis on development oral formulations will persist.

Although oral delivery has become a widely accepted route of administration of therapeutic drugs. The gastro intestinal tract presents several formidable barriers to drug delivery. It is particularly for drugs that are susceptible to undergo degradation in the small intestine by gastric juice and enzymes. Therefore to achieve as well as to maintain the steady state drug concentration within the therapeutically effective range needed for treatment, it is often necessary to take this type of drug delivery system several times a day. This results in a significant fluctuation in drug level.

Recently several technical advancements have been made. They

have resulted in the development of new techniques for drug delivery. These techniques are capable of controlling the rate of drug delivery, sustaining the duration of therapeutic activity and / or targeting the delivery of drug to a tissue.

***Sustained release* is** used to describe a pharmaceutical dosage form formulated to retard the release of a therapeutic agent such that its appearance in systemic circulation is delayed and / or prolonged and its plasma profile is sustained in duration. The onset of its pharmacologic action is often delayed and the duration of its therapeutic effect is sustained.

Controlled release its meaning that goes beyond the scope of sustained drug action. It also implies a predictability and reproducibility in the drug release kinetics. Which means that the release of drug ingredients from a controlled release drug delivery system proceeds a rate profile that is not only predictable kinetically but also reproducible from one unit to another(Chien et al.,1992).

Site targeted drug delivery system

Site targeted delivery system is proposed by Ringsdorf that it is constructed from a non immunogenic and biodegradable polymer back bone having three type of attached functional group. (1) A site-specific

targeting moiety that leads the drug delivery to the vicinity of a target tissue. (2) A solubilizer that enables the drug delivery system to be transported to and preferentially taken up by a target tissue. (3) A drug moiety that is covalently bonded to the polymer back bone through a spacer and contains a cleavable group that can be cleaved only by specific enzymes at the target tissue(Edith et al.,2002; Jain et al.,2001).

Colon target drug delivery system

Colon targeting is naturally of value for the topical treatment of diseases of colon such as Chron's diseases, ulcerative colitis, irritable bowel syndrome, amoebiasis and colorectal cancer.

This because conventional drug delivery system for the treatment of colon disorders are failing as the drug do not reach the site of action in appropriate concentration. Thus on effective and safe therapy of these colonic disorders using site specific controlled release formulation is an important and challenging task.

Colon specific delivery system is also gaining importance for the systemic delivery of protein, peptides, oligonucleotides and vaccines. This is because of the unprecedented rapid development of biotechnology and genetic engineering resulting in the availability of peptide and protein drugs at a reasonable cost. These drugs are destroyed and inactivated in

acidic environment of the stomach and /or by pancreatic enzymes in the small intestine. These drugs are usually administered by parenteral route which is inconvenient and expensive. The same problem is also faced by the above mentioned colonic disorder treating drugs.

To achieve successful colonic delivery a drug needs to be protected from absorption and or the environment of the upper GIT and then be abruptly release into the proximal colon in a controlled manner is considered the optimum site for colon targeted delivery of drug. It is also necessary to have a good knowledge about the anatomy and physiology of GIT for the targeting of the drug to the colon (Edith *et al.,* 2002; Jain *et al.*,2001,2008; Szente et al.,2007).

Anatomy and physiology of gastrointestinal tract

The gastrointestinal tract is divided into three parts stomach, small intestine and large intestine.

Stomach

Stomach is situated just below the diaphragm and liver. Its position however alters frequently i.e. it is pushed downwards with each inspiration and upward with each expiration. Its size varies according to several factors for some time after a meal the stomach is enlarge as food leaves the walls partially collapse. In adult stomach usually hold a volume of up to

1 liter to 1.5 liters.

Stomach is divided into three parts the fundus, body and pylorus. Fundus is the enlarged portion to the left and above the opening of esophagus into stomach. The body is the central part and pylorus is the lower portion. The curve formed by the upper right surface of the stomach is known as the lesser curvature and the curve formed by lower left surface is known as the greater curvature. The opening of stomach i.e. esophagus into stomach is controlled by cardiac sphincter and stomach into the first past of the small intestine is controlled by pyloric sphincter.

The stomach wall in made up of four layers serosa, longitudinal muscle layer, circular muscle layer, oblique muscle layer and sub mucosa layer. Gastric mucosa – The epithelial lining of the stomach is thrown into folds called rugates and marked by depression called gastric pits. Numerous coiled tubular type glands, gastric glands are found below the level of pits these glands secrete most of the gastric juice containing digestive enzymes and hydrochloric acid.

Small intestine

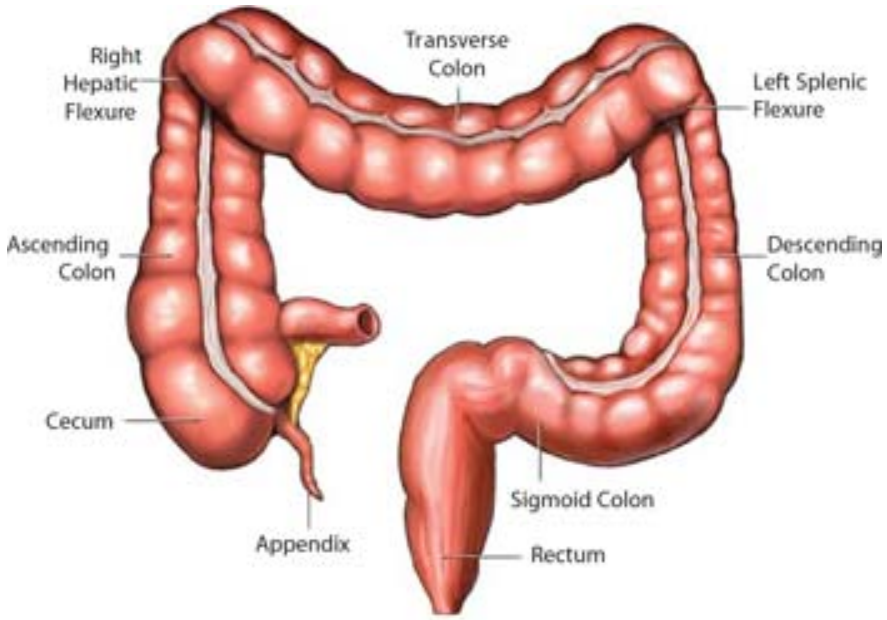
The small intestine is a tube measuring about 2.5cm (1 inch) in diameter and 6 meter (20feet) in length. It is a coiled loop till most of the abdominal cavity. The small intestine consists of three divisions the

duodenum, the jejunum and the ileum. The duodenum is the upper most division it is about 25cm (10 inches) long and is shaped roughly like the letter C. it is the shortest part of small intestine. Then starts the jejunum at the point where the tube turns abruptly forward and downward. It is about 2.5meter (8 feet). Then starts the ileum which is about 3.5 meter (12 feet) which is the longest part.

The intestinal wall is made up of outer serosa layer, longitudinal muscle layer, circular muscle layer and sub mucosa layer. The inner epithelial lining has circular plicae (fold) that have many tiny projection called villi. Each villus contains an arteriole, venule and lymph vessel. The presence of villa and microvilli increases the surface area of the small intestine making this organ the main site of digestion and absorption. Mucus secreting globlet cells are found in large number on villi and incrypts. The intestinal crypts serve as a site of rapid mitotic cell division.

Large Intestine

Fig. 1 Large Intestine



Anatomy of Large Intestine

It is the lower part of the alimentary canal which is larger in diameter than small intestine. Its length is about 1.5 to 1.8 meter (5 to 6 feet). Its average diameter is about 6cm (2 ½inches) but this decreases towards the lower end of the tube.

The large intestine is divided into cecum colon and rectum. Cecum is the first past of 5 to 8cm length. Colon which is divided into ascending, transverse, descending and sigmoid colon.

Ascending colon – lies in a vertical position on the right side of the abdomen, extending up to the lower border of the liver. The ileum joints the large intestine at the junction of the cecum and ascending colon, the place of attachment resembling the letter T.

Transverse colon – passes horizontally across the abdomen below the liver, stomach and spleen the transverse colon extends from the hepatic flexure to the splenic flexure the two points at which the colon bends on itself to form 90 degree angles.

The descending colon – lies in the vertical position on the left side of the abdomen, extending from a point below the stomach and spleen to the level of the iliac crest.

The sigmoid colon – it is the portion of the large intestine that courses down ward below the iliac crest is called sigmoid meaning “S”. It describes an S shaped curve. The lower part of the curve joint the rectum bends towards the left.

Rectum

The last 17 to 20cm (7 or 8 inches) of the intestinal tube is called the rectum. The terminal inch of the rectum is called the anal canal. Its mucous lining is arranged in numerous vertical folds known as anal columns each of which contains artery and a vein.

The intestinal wall consist of serosa, longitudinal muscle layer, circular muscle layer and sub mucosa layer. The longitudinal muscles are grouped into tube like strips called taeniae coli and the circular muscles are grouped into ring that produce pouch like haustra between them. The mucous glands present in sub mucosa layer which produce the lubricating mucus that coats the feces as they are formed.

Blood supply to GIT

Arterial supply to stomach is mainly by celiac artery and to small intestine by superior mesenteric artery and to large intestine by superior and inferior mesenteric artery. Superior supplies to the caecum, ascending and most of transverse colon, inferior supplies to the remainder of the colon and proximal past of rectum. Internal iliac arteries supply to the distal section of the rectum and the anus.

Function of the stomach

It serves as a reservoir, storing food until it can be partially digested and moved faster along the gastro intestinal tract. It secretes gastric juice containing acid and enzymes to aid in the digestion of food. Through contraction of its muscular coat it churns the food breaking into small particles and mixing them well with the gastric juice. In time, it moves the gastric content in to the duodenum. It secretes intrinsic factor. It produces

the hormones gastrin. Which helps regulate digestive functions and ghrelin which increases appetite. It protects the body by destroying pathogenic bacteria swallowed with food or with mucus from the respiratory track. It carries on a limited amount of absorption of certain drugs, water, alcohol and some short chain fatty acid found in butter or milk fat.

Function of small intestine

The main function of small intestine is digestion and absorption.

Digestion in small intestine- The acidic chyme from the stomach enters into the duodenum there it mixes with the alkaline intestinal juice called succus entericus and the alkaline secretion from the liver and pancreas. The enzymes of intestinal juice are enterokinase, Erepsin sucrase, maltase and lactase.

Absorption in small intestine- The absorption of digested food occurs in small intestine through villi. The villi has a centeral lymphatic vessel called lacteal fats are absorbed into lacteal, a net work of capillaries surrounding the lacteal, digested products of carbohydrates and proteins are absorbed into these capillaries they are carried to liver by portal vein.

Function of Colon

The major function of the colon is the consolidation of the intestinal contents into faces by the absorption of water and electrolytes and to store the faces until excreted. The absorptive capacity is very high; each day about 200ml of fluid enters the colon through the ileocecal valve from which more than 90% of the fluid is absorbed. In a healthy human colon sodium and chloride ions are usually secreted, on average it has been estimated that colon contain only about 220gm of wet material equivalent to just 35gm of dry matter. The majority of the dry matter is bacteria.

Activity in the colon can be divided into segmenting and propulsive movement segmenting movements caused by circular muscle and causing the appearance of the sac like haustre. Predominate and result in mixing of the lumenal contents significant propulsive activity associated with defecation and effected by longitudinal muscle is less common and occurs at an average of three or four times daily (Gary *et al*.,2004).

Colonic microflora

A large number of anaerobic and aerobic bacteria are present throughout the entire length of the human GIT. The upper region of the GIT has a very small number of bacteria and predominantly consists of

Gram positive facultative bacteria. The concentration of bacteria in the stomach is usually less than 10^3 colony forming units / ml (CFU/ml) and the most commonly isolated species are streptococci, staphylococci, lactobacilli and various fungi under normal conditions the microflora of the proximal small bowel are similar to those of the stomach. The bacterial concentration being 10^3-10^4 CFU/ml is found. In the distal part of the small intestine, a higher concentration of anaerobic bacteria is found. The lower ileum has a bacterial concentration of 10^7 - 10^8 CFU/ml and the distal ileum usually contains bacteria similar to those found in the colon. In the ileocecal sphincter, the bacterial concentration increase dramatically. The concentration of bacteria in the human colon is 10^{11} - 10^{12} CFU/ml. The bacterial flora of the colon is predominantly anaerobic and composed of more than 400 strains. The anaerobic bacteria outnumber the aerobic by a factor of 10^3 to 10^4. The most important anaerobic bacteria are Bacteroids, Bifidobacterium, Eubacterium, Peptococcus, Peptostreptococcus, Rumino coccus, Propionibacterium and clostridium. Important facultative bacteria in the large intestine are Escherichia coli and lactobacillus. The rate of microbial growth is greatest in the proximal area because of high concentration of energy source. The principle source of nutrition for the colonic micro organism is carbohydrate arriving in intestinal chime. A large

number of compound ingested orally are metabolized by gut bacteria. The most important metabolic reaction carried out by intestinal bacteria is

Hydrolysis of - Glycosides, sulphate esters, amides, esters, sulphamates and nitrates.

Reduction of - C = C, azo bond, nitro groups aldehydes, ketones, alcohols and N oxides

Dehydroxylation, Decarboxylation, Dehalogenation of O- alkyl group and N alkyl group, Dehalogenation, Desamination, Heterocyclic ring fission, Acetylation, Esterification.

These metabolic actions of the micro flora are influenced by factors like age, gastrointestinal disease, and intake of drug and fermentation of dietary residues. These can lead to inactivation of drug or the enhancement of the action and side effects of the drugs. (Watts *et al.,* 1997 ; Jain *et al.,*2001)

pH in the GIT

The pH of the GIT is subjected to both inter and intra subject variations. Over view of the pH of GIT.

Oral cavity - 6.2 -7.4

Oesophagus - 5.0 -6.0

Stomach	-	Fasted condition 1.5 -2.0
		Fed condition 3.0 -5.0
Small intestine	-	Jejunum – 5.0 -6.5
		Ileum – 6.0 – 7.5
Large intestine	-	Right colon - 6.4
		Mid and left colon – 6.0 -7.0

Radiotelemetry has been used to measure the gastro intestinal pH in healthy human subject. The highest pH level 7.5 ± 0.5 were found to be in the terminal ileum. On entry into the colon the pH dropped to 6.4 ± 0.6. The pH in the mid colon was found to be 6.6 ± 0.8 and in the left colon 7.0 ± 0.7. There is a fall in pH on entry into the colon due to the presence of short chain fatty acids. Arising from bacterial fermentation of polysaccharides. It is also reduce the pH in disease eg – in a group of 7 patients with untreated ulcerative colitis the mean pH in the proximal colon was 4.7 ± 0.7 where as in a group of 5 patients receiving treatment it was 5.5 ± 0.4(Gary et al.,2004; Jain et al.,2001;Sarasija et al.,2000).

Transit of material in the GIT

Gastric emptying of dosage form is highly variable and depends primarily on whether the subject is fed of fasted and on the

properties of the dosage form such as size and density. The arrival of an oral dosage form at the colon is determined by the rate of gastric emptying and the small intestinal transit time. The transit times of small dosage form in GIT is

Stomach	-	<1 (fasting) hours
	-	>3 (Fed) hours
Small intestine	-	3-4- hours
Large intestine	-	20-30 hours

The presence of food generally increase gastric residence and in some cases with regular feeding dosage form have been shown to reside in the stomach for period in excess of 12 hours (Rubinstein et al.,1991; Jain.N.K et al.,2001;Sarasija et al.,2000). Small intestine transit is surprisingly constant at 3- 4 hours and appears to be independent of the dosage form and the subjects fasted or fed state. Therefore a dosage form could take from a little as 4 hours to longer than 12 hours to arrive at the colon following oral administration. However the movement of material through the colon is slow and tends to be highly variable and influenced by a number of factor such as diet, dietary fiber content, mobility, stress, disease and drugs. In healthy young and adult male dosage forms such as capsule and tablet pass through the colon in

approximately 20-30hours. Although the transit time of a few hours to more than 2 days can occur.

The effect of capsule size and density on colonic transit has been investigated. Capsule with a density of 1.1gm/cm^3 and a volume of 0.3, 0.8 and 1.8 cm^2 and capsule with a volume of 0.8cm^3 and a density of 0.7 and 1.5 $gram^3$ were tested. Capsule transit through the ascending colon was not affected by density and although there was a tendency for the transit rate to increase with volume this effect was not significant Dependency of dosage form dimension on colonic transit is demonstrated in a study which compare the colonic transit of 3mm, 6mm, 9mm and 12mm tablet in 2 out of 8 subject 6mm move a head of 3mm tablet and in all subject 9mm tablet move a head of 6mm tablet 20mm tablet move ahead of 6mm tablet 20mm tablet move ahead of 6mm tablet in only 3 of the subject. The lower degree of separation between 6mm and 12mm compare to 6mm and 9mm was explained by the fact that while the 9mm tablet has a thickness and diameter compare to the 6mm only the diameter of the 12mm tablet was change which perhaps suggests that rate of colonic transit of tablet is volume dependent(Rubinstein et al.,1991;1992;Edith et al.,2002).

The result suggested that smaller units travel through the colon

more slowly than larger ones. Hence additional retention of a dosage form within the colon could perhaps be achieved by the use of a multi particulate formulation rather than a large single unit thus ensuring that it does not pass too rapidly through the colon and be excreted before the entire drug has been released.

Dietary fiber influence greatly the colonic motility, dietary fiber increase fecal weight partly by retention of water and partly by increasing bacterial mass and reduces colonic transit times eg. Additional of 20gm/day of brain to the diet of group of healthy subjects increased stool weight by 127% and reduced whole gut transit by 73 ± 24 hour to 43 ± 7 hours. Ingestion of food has been found to stimulate colonic activity in what is termed the gastro colonic response the effect of eating a meal on the colonic transit of radio labeled tubes shows ingestion of food accelerates the movement of tablet through the ileocecal junction into the colon.

Diseases affect colonic transit has important implication for drug delivery diarrhea increase colonic transit and constipation decrease colonic transit. However in most disease condition transit time appears to remain reasonable constant (Sarasija et al.,2000;Dysseler et al.,1995).

Drug absorption in the GIT

The unionized lipid soluble drugs are readily absorbed from stomach and intestine. The acidic drugs are unionized in stomach so are absorbed from the stomach and basic drug are ionized in the stomach so are absorbed in the small intestine.

Absorption of drug from the colon is limited by a number of barriers in lumen itself specific and non specific drug binding can occur with dietary compound and products released from colonic bacteria. This drug binding might facilitate longer colonic residence time and hence more enzymatic or environmental degradation.

The mucus layer at the epithelial surface presents a formidable physical barrier because of specific and non specific drug binding. As a corollary to drug-mucus binding drug-mucus repulsion or expulsion could also act to retard the drug from reaching the epithelial surface. The space between the mucus layer and epithelial cell i.e. the unstirred water layer present another barrier to colonic absorption particularly for lipophilic drugs. Drug that intend to pass from the epical to basolateral surface of epithelial barrier must do so by passing through either colonocytes (the transcellular route) or between adjacent colonocytes (the paracellular route). Receptor mediated endocytosis and fluid – phase endocytosis

(pinocytosis) represent two active transport pathway which could result in transcellular drug delivery. Paracellular transport may be the most promising means of general drug absorption in the colon.

The positive aspect for drug absorption is the prolonged residence time in the colon. The factor influencing the residence time are likely to affect the absorption through the colon can be increase by using absorption enhancer (Jain et al.,2001;Chourasia.,2004) .

Role of absorption enhancers

The permeability of the epithelium to drug can be modified by using chemical enhancers which promote absorption. The enhancer increase transcellular and paracellular transport by any one of the following three mechanisms.

1. By disruption of inter cellular occluding junction complex formulation to open the paracellular route.
2. By modifying epithelial permeability via denaturing membrane proteins or modifying lipid protein interaction.
3. By disrupting the integrity of lipid bilayer of colonic enterocytes.

Adsorption enhancers used in colonic drug delivery

a. Non steroidal anti-inflammatory agent indomethacin, salicylates.

b. Calcium 10n chelating agents – Ethyl enediamineratra acetic acid.

c. Surfactants – polyoxyethylene lauryl ether

d. Saponins

e. Bile salt – Taurocholate, Glycocholate

f. Fatty acid – Sodium caprate, Sodium caprylate, Sodium laurate, Sodium oleate.

g. Mixed micelles

Monoolein – taurochalate, oleic acid taurocholate, oleic acid – polyoxyethylene hydrogenated castor oil, oleic acid – glycocholate (Jain et al.,2001).

Diseases in colon

Diseases found in colon are Chron's diseases, ulcerative colitis, irritable bowel syndrome, amebiasis and colorectal cancer.

Colon cancer

Colorectal carcinoma is the second leading cancer killer and third most common cancer overall. The incidence of colorectal cancer is second for men next to lung cancer and second in women next to breast cancer. Colorectal cancer occurs most frequently after the age of 50; approximately 7% are seen in patients less than 40 years of age, about 30-50% of patients have advanced disease when they are diagnosed.

Incidence of colorectal malignancies is reported to be high in USA, Northern and Western Europe, where as an increasing incidence is

observed in Asia, Africa and South America, surgery and systemic chemotherapy is the major therapeutic option and radiation mod of therapies are also of great important.

Predisposing factors

Specific causes of colorectal cancer are unknown but environmental nutritional, genetic, familial factors and pre-existing diseases have been found to have some association. It has been seen that risk increases with consumption of animal fat and red meats while it decreases with consumption of fiber, vitamins 'A' & 'D', calcium and folate.

Symptomatology

Adeno carcinomas of the colon and rectum grow slowly and may be presents for as long as five years before symptoms appears. Symptoms shows are change in bowel habits, fecal blood, rectal bleeding, abdominal pain, unexplained anemia or weight loss and fatigue.

Pathology

The adenomas carcinoma sequence is established as the origin of nearly all colonic carcinomas from adenomas. Severe epithelial dysplasia is the precursor to invasive carcinoma with critical minimum size of 5mm with in an adenoma. The gross morphological feature of adenocarcinoma in the large bowel depends on the location of tumor carcinomas of

proximal colon tend to be large and bulky. In the more distal colon and rectum tumors involve a greater circumference of the bowel producing an annular constriction or napkin ring appearance.

Fig. 2 Colon Cancer

Classification

According to Maruyoma classification is based on microscopic criteria.

Borrmann type I

Circumscribed, solitary polypoid, in part shaggy surface.

Borrmann type II

Ulcerated carcinoma with elevated margins, this is the most common type and represents approximately two third of all colon carcinomas.

Borrmann type III

Ulcerative carcinoma with elevated or diffuse margins (inter mediate type).

Borrmann type IV

Diffuse infiltrating (linitis plastic) infiltration with hardening often servers stenosis occurs. This occurs typically in younger patients and carrier a poor prognosis. Secondary colon carcinoma is frequently of this type.

Carcinomas of the large bowel are predominantly adenocarcinoma 50% occur in the rectum and sigmoid colon and 35% in the right colon. In 15% of tumors large lakes of mucin contain scattered collection of tumor cells. Cancer other than adeno carcinoma comprises less than 5% of malignant tumors of large bowel and includes squamous cell carcinoma and colacogenic or transitional cell carcinoma (Manorama et al.,1997).

Drugs used in colon cancer (Subramanian et al.,2002)

5- Fluorouracil

Bevacizumab

Irinotecan

Centuximab

Panitumumab

Leucovarin

Oxaliplatin
Capecitabine } New drugs

Method for targeting drug into colon

Colonic targeting is advantageous in treating disease of colon, oral delivery of proteins and peptides these is achieved by following method(Jain et al.,2001; Edith et al.,2002; Chourasia *et al.,*2003).

1. Coating with pH dependent polymer
2. Timed release dosage forms
3. Delivery system based on the metabolic activity of colonic bacteria
4. Osmotic controlled drug delivery

1. Coating with pH dependent polymer

In this system drug are formulated into solid dosage form such as tablet, capsules, pellets and coated with pH sensitive polymer. The generally accepted view that pH of the human GIT increases progressively from the stomach (pH 1-2 which, increase to 4 during digestion) small intestine (pH 6-7) at the site of digestion and it increase to 7-8 in the distal ileum. The coating of pH sensitive polymer provides delayed release and protects the active drug from gastric fluid. The polymer used for colon

targeting however should be able to withstand the lower pH value of the stomach and of the proximal part of the small intestine and also be able to disintegrate of the neutral of slightly alkaline pH of the terminal ileum and preferable of the ileocecal junction. These processes distribute the drug throughout the large intestine and improve the potential of colon targeted delivery system (Chourasia *et a*l., 2002;Sarasija et al.,2000; Edith *et al*., 2002; Jain *et al.,*2001;Patel et al.,2008;Meissner et al.,2007;Ibekwe et al.,2008;Chiu et al.,1999) widely used polymer a methacrylic resins they are

- Eudragit L 100 (soluble at pH 6 or above)
- Eudragit S 100 (soluble at pH 7 or above)
- Hydroxy propyl methyl cellulose acetate Succinate (Aqoat AS – HF) (Soluble at pH 7 or above)
- Other commonly used polymer
- Eudragit L-30D (soluble at pH 5.6)
- Eudragit FS -30D (soluble at pH 6.8)
- Eudragit L 100 –SS (soluble at pH 5.5)
- Poly vinyl acetate phthalate (soluble at pH 5)
- Hydroxypropyl methylcellulose phthalate (soluble at pH 4.5-4.8)
- Hydroxy propyl methyl cellulose phthalate SD (soluble at pH 5.2)

- Hydroxyl propyl methyl cellulose phthalate SS (soluble at pH 5.4)
- Cellulose acetate trimetlliate (soluble at pH 4.8)
- Cellulose acetate phthalate (soluble at pH 5.0)

1. Timed release dosage forms

This approach is based on the principle of delaying the release of the drug until it enter into colon, although gastric emptying tends to be highly variable, small intestine transit time is relatively constant. So various attempts are made to prevent the release of drug in the stomach for that it is coated with polymer which is undissolved in the gastric pH, once it have left the stomach the formulation arrives at the ileocecal junction about 3 to 4 hour (Chourasia *et al.,* 2003; Sarasija *et al.,*2000; Edith *et al.,*2002; Jain *et al.,* 2001). For this purpose two types of systems are used.

Pulsing cap system

This delivery system consists of a capsule, half of which is non disintegrating and other half enteric coated. The enteric coat dissolves on entering the small intestine of a hydrogel plug stoppering the non disintegrating part swells at a rate determined by the degree of cross linking. After a predetermined time (5hours) the hydrogel plug swells so much that is becomes ejected from the non disintegrating bottom half of

the capsule. There by releasing the drug. It must note that the swelling of the hydrogel plug is pH independent (Chourasia *et al.,*2003; Sarasija *et al.,*2000; Edith et al.,2002; Jain *et al*.,2001).

Time clock

This delivery system has been exploited to release the drug in the colon. It is composed of a solid. Dosage form coated with a hydrophobic surfactant layer to which a water – soluble polymer is added to improve adhesion to the core. The outer layer redisperses in the aqueous environment in a time proportional to the thickness of the film and the core is then available for dispersion. In a study with human volunteers, it was shown that the lag time was independent of gastric residence time and hydrophobic film redispersing did not appears to be influenced by the presence of intestinal digestive enzymes or by mechanical action of the stomach (Chourasia et al.,2003; Sarasija *et al.,* 2000; Edith *et al.,*2002; Jain *et al.,*2001; Patel et al.,2008).

3 Delivery, system based on the metabolic activity of colonic bacteria

The colonic bacteria carry out a variety of metabolic reaction and hydrolysis. Different strategies were used to target to the colon based on

these action. The main feature of this system is their site specificity. They are *Prodrug(Friend et al.,1984;1985; Dysseler et al.,1995;Harboe et al.,i989;Mladenovaka et al.,2007).*

A well known colon specific prodrug is sulfa salazine sulfa sulazine is a conjugate of sulphapyridine and 5 amino salicylic acid (5-ASA) with the molecules linked by an azo bond (-N = N-) in the treatment of inflammatory bowel diseases – sulpha- salazine breaks up as 5-ASA and sulphapyridine by azo reductase(Dhaneshwar et al.,2007;MacNeil et al.,1990; Tozaki et al.,2002).

Another prodrug approach is glycosides glycosidases are a prominent group of enzymes produced by the intestinal microflora. The major glycosidases found in human faces are β-D galactosidase, α – L-arabinofuranosidase, β-D- xylo-pyranosidase and β-D–glucosidase prodrug of prednisolone, dexamethasone, hydrocortisone and fludrocortisones with β-D – galactosides and β-D-glucosides were prepared which are larger and hydrophilic and thus are not absorbed from small intestine.

Another approach is dextran ester prodrugs of naproxen were synthesized and tested for their drug release in homogenates of various segments of the pig GIT. Drug release was found precede 15-17 times

faster in caecum and colon homogenates than in homogenates of the small intestine. The drug release was attributed to the initial depolymerisation of dextran chains by dextranases of the pig colonic bacteria and the result and fragments served as substrates for esterases and other hydrolases(Jonson et al.,1966; McLeod et al.,1993;1994).

Anti inflammatory glucocorticoids do not process carboxylic acid groups and must be chemically transformed in order to reach with dextran. Dexamethasone and methyl prednesolone were attached to dextran using specific as a spacer (Chourasia *et al.,* 2003; Sarasija *et al.,* 2000; Sinha *et al.,* 2003; Edith *et al.,* 2002; Jain *et al.,*2001).

Azo polymers

Poly styrene and hydroxyethylmethacrylate cross linked with divinylazobenzene have been used for colon delivery. Hydrophilic azo polymers containing different ratios of methylmethacrylate and hydroxethylmethacrylate (HEMA) content showed greatest susceptibility to colonic degradation similar result have been seen with azo containing polyamides and poly urethane. The swelling of the polymer in pH-dependent and swells in the colon to allow access to bacterial azo reductase enzymes (Kimura et al.,1992; Chourasia *et al.,* 2003; Sarasija *et*

*al.,*2000; Sinha *et al.,* 2003; Edith *et al.,* 2002; Jain *et al.,*2001; Dhaneshar et al.,2007).

Hydrogel

The hydrogel are used for the delivery of peptide and protein drugs. The hydrogel contain acidic comonomers and enzymatically degradable azo aromatic cross links. In the acidic pH of the stomach the gels have a low degree of swelling which protect the drug against degradation by digestive enzymes. As the gels pass down the GIT, the degree of swelling increases. On entering the colon the gels reach a degree of swelling which make the cross links accessible to enzymes (azoreductases) or mediators (electron carriers). The cross links are then degraded and the drug is released from the disintegrated gels(Bronsted et al.,1991;Chin et al.,1999; Vervoort et al.,1998; Chourasia et al.,2003;Sarasija et al.,2000; Sinha *et al*.,2003; Edith et al.,2002; Jain *et al.,*2001).

4 Osmotic controlled drug delivery

The osmotic controlled drug delivery system can be used to target the drug locally to the colon for the treatment of disease or to achieve systemic absorption that is otherwise unattainable. The OROS –CT system can be single osmotic unit or may incorporate as many as 5-6 push-pull units each 4mm in diameter, encapsulated within a hard gelatin

capsule. Each bilayer push pull unit contain an osmotic push layer and a drug layer both surrounded by a semi permeable membrane. An orifice is drilled through the membrane next to the drug layer. Immediately after the OROS-CT is swallowed the gelatin capsule containing the push pull units dissolves. Because of its drug – impermeable enteric coating each push pull units is prevented from absorbing water in the acidic aqueous environment of the stomach and hence no drug delivered. As the unit enter the small intestine the coating dissolve in this higher pH environment (pH > 7), water enters the unit causing the osmotic push co0mpartment to swell and concomitantly creates a flowable gel in the drug compartment swelling of the osmotic push compartment forces drug gel out of the orifice at a precisely controlled by the rate of water pass through the semipermiable membrane.

By using the above mentioned four method of colon targeting different formulations are made they are pellets, beads, matrix tablet, nanoparticles, microspheres etc. for my study microspheres has been selected because it can be prepared using normal lab equipments and do not require sophisticated instruments and can done in a less expensive manner (Chourasia *et al.,*2003).

Microspheres

Microspheres are defined as a spherical particle which contains a core substance. They have a characteristically free flowing powder consisting of proteins or synthetic polymer which is biodegradable in nature and ideally having a particle size less than 200µm (Vyas *et al.,*2002).

Advantage of microsphere

- Longer duration of action
- Control of content release
- Increase of therapeutic efficiency
- Protection of drug
- Reduction of toxicity
- Biocompatibility
- Sterilizability
- Relative stability
- Water solubility or dispersability
- Targetability

General method of preparation

Various technique are used and the selection is depends upon the nature of polymer used, amount of drug used and the duration of

therapy.

Types of methods

1. Single emulsion technique
2. Double emulsion technique
3. Polymerization

a. Normal polymerization

b. Interfacial polymerization

4. Phase separation coacervation
5. Spray drying and spray congealing
6. Solvent extraction

1. Single emulsion technique

Natural polymer is prepared by this method. The polymer is dissolved or dispersed in an aqueous medium and is added to non aqueous medium (oil) to form emulsion. Then crosslinking should be achieved by either heat or chemical agent so formed microsphere are washed and dried.

2. Double emulsion techniques

Preparation involves the formation of multiple emulsion double of type W/O/W. It is suited for water soluble drugs, peptides, proteins

and vaccines. Both natural and synthetic polymers are used. The drug is dissolved in an aqueous medium and added to a lipophilic organic continuous phase stir it to form first emulsion. This is added to the aqueous solution of poly vinyl alcohol (PVP). This result in double emulsion. The emulsion is subjected to solvent removal by either solvent evaporation or solvent extraction then filter washed and dried.

3. Polymerization techniques

These techniques consist of two type normal and interfacial polymerization

Normal polymerization

Normal polymerization process is carried out by using different technique

Bulk polymerization

Suspension precipitation polymerization

Emulsion/ Miceller polymerization

Bulk polymerization

Monomers or mixture of monomers along with drug and initiator is usually heated for polymerization. Catalyst is added to accelerate the reaction. The so formed polymer are molded or fragmented as microsphere.

Suspension precipitate polymerization

Polymerization is carried out by heating the monomers or mixture of monomers with drug as droplets in aqueous phase, vigorous agitation and heating of the aqueous phase with droplets form polymerization, which is then washed dried to get microsphere emulsion polymerization.

Differ from suspension polymer due to the presence of initiator in the aqueous phase which later on diffuses to the surface of the micelles or the emulsion globules.

Interfacial polymerization

It occurs by the reaction of various monomers at the interface between two immiscible liquid phases. The monomers present in the two phases are diffuse rapidly and polymerize rapidly at the interface.

4. Phase separation coacervation technique

Phase separation method is specially designed for preparing the reservoir type of the system / matrix type system. The process is based on the principle of decreasing the solubility of the polymer in the organic phase to affect the formation of the polymer rich phase called the coacervates. The coacervates can be brought about by addition of the third component that are salt addition, non solvent addition, addition of in compatible polymer and change in pH.

5. Spray drying and spray congealing

Method is based on the drying of the mist of the polymer and drug in the air. Removal of solvent is done by two method spray drying and spray congealing soldering. The rate of solvent removal is depending upon the temperature, pressure, solubility, the polymer, the solvent and the dispersion medium.

6. Solvent extraction

Solvent extraction method is used for the removal of organic phase in which the microspheres is formed by using worker as solvent extraction. The rate of solvent removal depends on the temperature of water, ratio of emulsion, volume of the water and the solubility of the polymer, this process decreases the hardening time of the microspheres(Onishi et al.,2008; Chourasia et al.,2004;Paharia et al.,2007;Anande et al.,2008; Ziyaur et al2006).

REVIEW OF LITERATURE

Chandran. *et al.,* (2008) designed and developed microspheres of indomethacin with pH and transit time dependent release properties for achieving targeted delivery to the colon. Microspheres containing varying proportions of ethyl cellulose and Eudragit (L100 or S100) either alone or in combination were prepared using an oil-in-oil emulsion-based solvent evaporation technique. microspheres with good physical properties, high drug loading (70-80%) and entrapment efficiency (70-85%). The lag time in the initial release depended on the proportion of pH-sensitive polymer Eudragit, while the duration of indomethacin release from microspheres was found to be directly proportional to proportion of the total polymer. Thus, a pH- and time-modulated sigmoidal release pattern could be observed in optimized formulations with less than 10% drug release in 4-6 hrs followed by controlled release extending up to 14-16 hrs.

Onishi *et al.,* (2008) developed a targeted delivery system for inflammatory bowel disease (IBD), Eudragit L100 (EuL)-coated chitosan (Ch)-succinyl-prednisolone (SP) conjugate microspheres (Ch-SP-MS/EuL), were designed and examined *in vivo* for efficacy and toxicity. By synthesis of the conjugate by carbodiimide coupling of Ch and SP, conversion into microspheres (Ch-SP-MS), and coating of Ch-SP-

MS

with EuL. Experimental colitis was induced by instillation of 2,4,6-trinitrobenzenesulfonic acid (TNBS) into the colon in rats. Efficacy was dose-dependent and the greatest in the order Ch-SP-MS/EuL>Ch-SP-MS>prednisolone (PD) alone, and Ch-SP-MS/EuL showed excellent recovery of colitis states. Toxicity was the greatest in the order PD>>Ch-SP-MS>Ch-SP-MS/EuL. Ch-SP-MS and Ch-SP-MS/EuL reduced significantly the thymic atrophy caused by PD. It was demonstrated that Ch-SP-MS/EuL enhanced effectiveness of PD and reduced toxic side effects of PD greatly. Also, these results established the prediction by previous *in vitro* and *in vivo* studies.

Kaur *et al.,* (2008) developed prednisolone (PDS) beads which were coated sequentially with (i) innermost hydrophobic layer of Eudragit((R)) RS/RL, (ii) middle drug release-triggering layer of chitosan, organic acid and Eudragit (R)) RS/RL, and (iii) outermost enteric coating layer. Continuous dissolution studies were carried out in artificial simulated gastric fluid with and without beta-glucosidase.. Succinic acid provided the fastest rate of release in the colonic fluid compared to citric, tartaric or malic acid. The results of plasma pharmacokinetic studies in Sprague-Dawley rats showed that the developed system provided a significant delay (T(max) 9.3h) in the absorption profile of PDS compared with simple enteric-coated (T(max) 4h) or powder (T(max) 1h) formulation that

was taken as proof for the colon-targeted delivery.

Patel *et al.,* (2008) developed a time- and pH-dependent system for delivering mesalamine to the colon. The system consists of the core tablet of mesalamine which is compression coated with hydroxypropyl methylcellulose (HPMC K4M) (time-dependent factor). This is then coated with pH-dependent polymer Eudragit (R) L100. The simplex lattice design was adopted to optimize the independent variables i.e. amount of HPMC (X1), dextrose (X2) and polyvinyl pyrollidone (PVP) (X3) and to study their effect on the dependent variables i.e. lag time and time for 50% drug dissolution (t50). The results of the linear interactive model and graphical representation revealed that as the amount of HPMC increases, the lag time and t50 value also increases and as the amount of dextrose and PVP were increased the lag time and t50 value decreases.

Anande *et al.,* (2008) developed cyst-targeted novel concanavalin-A (Con-A) conjugated mucoadhesive microspheres of diloxanide furoate (DF) for the effective treatment of amoebiasis. Eudragit microspheres of DF were prepared using emulsification-solvent evaporation method. Formulations were characterized for particle size and size distribution, percetnage drug entrapment, surface morphology and *in vitro* drug release in simulated gastrointestinal (GI) fluids. Eudragit microspheres of DF were conjugated with Con-A. IR spectroscopy and DSC were used

to confirm successful conjugation of Con-A to Eudragit microspheres while Con-A conjugated microspheres were further characterized using the parameters of zeta potential, mucoadhesiveness to colonic mucosa and Con-A conjugation efficiency with microspheres. All the microsphere formulations showed good % drug entrapment (78+/-5%). Zeta potential of Eudragit microspheres and Con-A conjugated Eudragit microspheres were found to be 3.12+/-0.7mV and 16.12+/-0.5mV, respectively. Attachment of lectin to the Eudragit microspheres significantly increases the mucoadhesiveness and also controls the release of DF in simulated GI fluids. Gamma scintigraphy study suggested that Eudragit S100 coated gelatin capsule retarded the release of Con-A conjugated microspheres at low pH and released microspheres slowly at pH 7.4 in the colon.

Ibekwe *et al*., (2008) assessed *the in vivo* targeting performance of a novel colonic delivery coating comprising a mixture of pH-responsive enteric polymer (Eudragit S) and biodegradable polysaccharide (resistant starch) in a single layer matrix film. Tablets (radio-labelled) were film-coated with the dual-mechanism coating and administered in a three-way crossover study to eight healthy volunteers (i) without food, (ii) with breakfast or (iii) 30 min before breakfast. The site of intestinal disintegration was assessed using gamma scintigraphy. The coated

tablets were able to resist breakdown in the stomach and small intestine. Consistent disintegration of the dosage form was seen at the ileocaecal junction/large intestine. The site of disintegration remained unaffected by feeding. The dual-mechanism (pH/bacterial) coating provides colon-specificity.

Ravi *et al.,* (2008) developed a novel colon targeted tablet formulation using natural polysaccharides such as chitosan and guar gum as carriers and diltiazem hydrochloride as model drug. The prepared blend of polymer-drug tablets were coated with two layers, inulin as an inner coat followed by shellac as outer coat. *In vitro* studies revealed that the tablets coated with inulin and shellac have controlled the drug release in stomach and small intestinal environment and released maximum amount of drug in the colonic environment. Among the polymers used, chitosan was found to be the suitable polymer for colon targeting. The study revealed that polysaccharides as carriers and inulin and shellac as coating materials can be used effectively for colon targeting of drugs for treating local as well as systemic disorders.

Wei *et al.,* (2007) developed colon-targeted drug delivery systems for 5-fluorouracil using pectin combined with ethylcellulose as a film coat with fluidized bed coater. Pellets (0.8-1.0 mm in diameter) containing 40%

5-fluorouracil and 60% microcrystalline cellulose were prepared by extrusion and spheronization. Film-coated pellets of 5-fluorouracil containing various proportion of pectin and ethylcellulose (Surelease) were prepared and subjected to *in vitro* drug release. The results of the study show that the formulation of pectin to Surelease 1:2, w/w is most likely to provide targeting of 5-fluorouracil for local action in the colon, as it released only 4.1 +/- 0.4% of the drug in the simulated gastric and small intestinal conditions, and it released 85.0 +/- 0.3% of 5-fluorourail in simulated colonic fluids at the end of 24 h. Differential scanning calorimetric study indicated no possibility of interaction between 5-fluorouracil and pectin or other excipients used in the coated pellets.

Mundargi *et al.,* (2007) developed colon targeted drug delivery systems for metronidazole (MTZ). Tablets were prepared using various polysaccharides or indigenously developed graft copolymer of methacrylic acid with guar gum (GG) as a carrier. Various polysaccharides such as GG, xanthan gum, pectin, carrageenan, beta-cyclodextrin (CD) or methacrylic acid-g-guar (MAA-g-GG) gum have been selected and evaluated. The prepared tablets were tested *in vitro* for their suitability as colon-specific drug delivery systems. Further, *in vitro* release was performed in the dissolution media with rat caecal contents. Results

indicated an enhanced release when compared to formulations studied in dissolution media without rat caecal contents, because of microbial degradation or polymer solubilization. The nature of drug transport was found to be non-Fickian in case of uncoated formulations, whereas for the coated formulations, it was found to be super-Case-II. Statistical analyses of release data indicated that MTZ release is significantly affected by the nature of the polysaccharide used and enteric coating of the tablet. Differential scanning calorimetry indicated the presence of crystalline nature of drug in the formulations.

Dhaneshwar *et al.,* (2007) developed mutual azo prodrug of 5-aminosalicylic acid with l-tyrosine was synthesized by coupling l-tyrosine with salicylic acid, for targeted drug delivery to the inflamed gut tissue in inflammatory bowel disease. The structure was confirmed by elemental analysis, IR and NMR spectroscopy. *In vitro* kinetic studies in rat fecal matter showed 87.18% release of 5-aminosalicylic acid with a half-life of 140.28 min, following first order kinetics. Therapeutic efficacy of the carrier system and the mitigating effect of the azo conjugate were evaluated in trinitrobenzenesulfonic acid-induced experimental colitis model. Myeloperoxidase activity was determined by the method of Krawisz *et al.,* The synthesized prodrug was found to produce comparable mitigating

effect as that of sulfasalazine on colitis in rats.

Meissner *et al.,* (2007) developed a pH-sensitive microspheres loaded Enoxaparin using Eudragit P4 135F that dissolves at pH>7.2. Particle preparation was based on a double emulsion technique with either solvent extraction or evaporation. Solvent evaporation led to higher entrapment rates (evaporation: 70.1+/-9.9%; extraction: 46.5+/-6.4%). As expected, *in vitro* drug release was found to be strongly pH-dependent; LMWH was retained in microspheres at pH<6 (<20% release within 4h) whereas a fast drug release was obtained at pH 7.4 . These microspheres represent a promising tool for the selective oral delivery of heparin to the colon, especially interesting in the treatment of inflammatory bowel disease.

Mladenovska *et al.,* (2007) developed chitosan-Ca-alginate microparticles for colon-specific delivery and controlled release of 5- aminosalicylic acid after peroral administration were prepared using spray drying.^{1}H NMR, FTIR, X-ray and DSC studies indicated molecularly dispersed drug within the particles with preserved stability during microencapsulation and in simulated *in vivo* drug release conditions. *In vitro* drug release studies carried out in simulated *in vivo* conditions in respect to pH, enzymatic and salt content confirmed the potential of the particles to release the drug in a controlled manner. Biodistribution studies of $^{(131}$ I) -5-ASA loaded chitosan – Ca-alginate microparticles, carried

out within 2 days after peroral administration to Wistar male rats in which TNBS colitis as induced, confirmed the dominant localization of 5-ASA in the colon with low systemic bioavailability.

Singh *et al.,* (2007) developed modified-release (MR) formulation technologies that are claimed to provide colonic delivery for a wide array of therapeutic molecules. These technologies either utilize a single or a combination of two or more physiological characteristics of the colon, which includes pH, microflora (enterobacteria), transit time, and luminal pressure. Accordingly, these technologies may be grouped under four distinct classes: pH-controlled (or delayed-release) system, time-controlled (or time-dependent) system, microbially controlled system, and pressure-controlled system. Among these, formulations that release drugs in response to colonic pH, enterobacteria, or both are most common and promising.

Szente *et al.,* (2007) developed colon specific drug delivery systems for diseases having circadian rhythm. Such circadian rhythm release drug delivery systems are designed to provide a plasma concentration--time profile, which varies according to physiological need at different times during the dosing period, i.e., mimicking the circadian rhythm and severity/manifestation of gastric acid secretion (and/or

midnight gerd). In general four primary approaches have been proposed for colon targeted delivery namely pH-dependent systems, time dependent systems, colonic microflora activated systems and prodrugs.

Paharia *et al.,* (2007) prepared and evaluated Eudragit-coated pectin microspheres for colon targeting of 5-fluorouracil (FU). Pectin microspheres were prepared by emulsion dehydration method. The yield of preparation and the encapsulation efficiencies were high for all pectin microspheres. Eudragit-coating of pectin microspheres was performed by oil-in-oil solvent evaporation method. Pectin microspheres and Eudragit-coated pectin microspheres were evaluated for surface morphology, particle size and size distribution, swellability, percentage drug entrapment, and *in vitro* drug release in simulated gastrointestinal fluids (SGF). The *in vitro* and *in vivo* drug release study is performed. The release profile of FU from Eudragit-coated pectin microspheres was pH dependent. In acidic medium, the release rate was much slower; however, the drug was released quickly at pH 7.4. It is concluded from the present investigation that Eudragit-coated pectin microspheres are promising controlled release carriers for colon-targeted delivery of FU.

Yongmei Xu *et al.,* (2007) prepared alginate-chitosan(ALG-CS)

blend gel beads based on Ca^{2+}or dual cross linking with various proportions of alginate and its chitosan. The dual crosslinkage effectively promoted the stability of beads under gastro intestinal tract conditions. The sustained release profiles of single and dual crosslinked gel beads loaded bovaine serum albumin (BSA), a model protein drug, were investigated in simulated gastric fluid(SGF), simulated intestinal fluid (SIF) and simulated colonic fluid (SCF). The dual crosslinked beads incubated in gastrointestinal tract conditions, the BSA cumulative release of ALG-CS mass ratios 9:1,7:3 and 5:5 respectively 2.35,1.96,1.76%(in SGF4h) 82.86%, 78.83,52.91% (in SIF 3h) and 97.84,96.81,87.26%(in SCF 3h), which suggested that the dual crosslinked beads have potential small intestine or colon site-specific drug delivery property.

Ziyaur *et al.,* (2006) prepared and evaluated the colon-specific microspheres of 5fluorouracil for the treatment of colon cancer. Core microspheres of alginate were prepared by the modified emulsification method in liquid paraffin and by cross-linking with calcium chloride. The core microspheres were coated with Eudragit S-100 by the solvent evaporation technique to prevent drug release in the stomach and small intestine. The microspheres were characterized by shape, size, surface morphology, size distribution, incorporation efficiency, and *in vitro* drug

release studies. The core microspheres sustained the drug release for 10 hours. The release studies of coated microspheres were performed in a pH progression medium mimicking the conditions of the gastrointestinal tract. Release was sustained for up to 20 hours in formulations with core microspheres to a Eudragit S-100 coat

Watts *et al.,* (2005) designed site-specific delivery of drugs in the gastrointestinal (GI) tract and, in particular, targeted release into the colonic region. A key area of application is the delivery of therapeutic agents for local treatment of lower GI diseases. The technology is based on the application of pH-sensitive coatings onto injection-moulded starch capsules. An extensive body of clinical data has been generated showing reliable *in vivo* performance of the capsules. In gamma-scintigraphy studies around 90% of targit capsules (n = 84) delivered their contents to the target site of the terminal ileum and colon. TARGIT-based products are in active clinical development for the treatment of conditions including inflammatory bowel diseases.

Varshosaz *et al.,* (2005) developed mesalazine (5-ASA) chitosan microspheres, colon-specific delivery system for crohn's disease and ulcerative-colitis. Coated chitosan microspheres were used for this purpose by an emulsion-solvent evaporation technique based on a

multiple w/o/w emulsion. Release of 5-ASA from microspheres was studied in different pHs 1.2,7.4,6.8 and 6.8 and in the presence of caecal contents of rat. Microspheres of chitosan with medium molecular weight and 1:1 core/coat that showed the greatest release of drug (near 80%) in the presence of caecal secretions with a zero-order mechanism, near zero percent in pH 1.2 after 2h, max 20% in pH 7.4 after 3h and near 60% in pH 6.8 after 8h seen suitable for site- specific delivery of 5 ASA *in vitro.*

Jain *et al.,* (2005) developed colon specific delivery system bearing flurbiprofen using various azo-aromatic polymers and pH-sensitive polymers are discussed *In vitro* dissolution studies showed that flurbiprofen bearing hard gelatin capsules, coated with these polymers released drug only in gastro intestinal fluid containing human fecal suspension, at pH 7.5. *In vivo* studies revealed that azo aromatic and pH-sensitive polymer coating disintegrate only in colon following 10h of oral administration. Hence these polymers can be successfully used to deliver drug at the colon.

Chourasia *et al.,* (2004) developed a novel formulation consisting of cross-linked microspheres of guar gum has been investigated for colon-targeted delivery of metronidazole. An emulsification method involving the dispersion of aqueous solution of guar gum in castor oil was used to prepare spherical microspheres. Shape and surface morphology of

the microspheres were examined using scanning electron microscopy. Particle size of the microspheres was determined using laser diffraction particle size analyzer. *In vitro* release rate studies were carried out in simulated colonic fluid (SCF) with and without in the presence of rat cecal contents, which showed improved drug release. A marked improvement in the drug release was observed in presence of cecal matter obtained after induction when compared to those without induction. *In vitro* release studies exhibited 31.23+/-1.49% drug release in 24 h in dissolution medium without rat cecal matter. However, the incorporation of 4% w/v cecal matter obtained after 6 days of enzymes induction increased the drug release to 96.24+/-4.77%.

Chourasia *et al.,* (2004) developed a multiparticulate system combining pH-sensitive property and specific biodegradability for colon-targeted delivery of metronidazole has been investigated. The multiparticulate system was prepared by coating cross-linked chitosan microspheres exploiting Eudragit L-100 and S-100 as pH-sensitive polymers. *In vitro* drug-release studies were performed in conditions simulating stomach-to-colon transit in presence and absence of rat caecal contents. By coating the microspheres with Eudragit pH-dependant release profiles were obtained. No release was observed at

acidic pH; however, when it reached the pH where Eudragit starts solublizing there was continuous release of drug from the formulation. Further, the release of drug was found to be higher in the presence of rat caecal contents, indicating the susceptibility of chitosan matrix to colonic enzymes released from rat caecal contents.

Momin *et al.,* (2004) developed colon targeted drug delivery systems for sennosides using guar gum as a carrier. Matrix tablets containing various proportions of guar gum were prepared by wet granulation technique using starch paste as a binder. The tablets were evaluated for content uniformity and *in vitro* drug release study as per BP method. The matrix tablets containing 50% of guar gum were found to be suitable for targeting of sennosides for local action in the colon. Compared to tablets having 30% and 40% of guar gum, those with 50% guar gum fewer amounts (5-8%) of drug release in upper GIT. These tablets with 50% guar gum released 43% and 96% sennosides with and without rat caecal fluids. This suggests the susceptibility of matrix to the colonic micro flora. When hydroxy propyl methylcellulose phthalate (10%) was used as a coat material on the matrix tablets, the initial loss of 5-8% sennosides in stomach could be completely averted. The results of our study indicates that matrix tablets containing 50% guar gum and coated with 10% hydroxy

propyl methylcellulose phthalate are most suitable for drugs like sennosides which are mainly active in the lower GIT.

El-Gibaly *et al.,* (2002) developed ketoprofen-loaded Zn-pectinate gel (ZPG) microparticles together with pectin/dextran mixtures in a tablet form, has been investigated, *in vitro*, using conditions chosen to simulate the pH and times likely to be encountered during transit to the colon. The results obtained implied that the release of ketoprofen from ZPG microparticles was greatly extended with the pectinate microparticles. The results also demonstrated that the untableted ZPG microparticles exhibited drug release profiles which were able to retard the release of ketoprofen in S.I.F. (pH 7.4) to be 5.28-37.82 times (depending on formulation parameters), lower than the conventional calcium pectinate beads. Therefore, this approach suggests that ZPG microparticles and their modified-release formulations are promising as useful controlled-release carriers for colon-targeted delivery of drugs.

Rasmane *et al.,* (2000) developed theophylline pellets coated with Eudragit NE30D aqueous dispersion, containing various pectin HM/Eudragit RL30D ionic complexes' using an uni-glatt fluidized-bed apparatus . Dissolution studies were then carried out on the coated pellets at pH 6.0, in absence and in presence of commercial pectinolytic

enzymes. The theophylline release from the coated pellets as slower in presence of the pectinolytic enzymes when the pectin content of the complexes is higher than 20.0%w/w. On the other hand, the effect of the enzymes induced an increase of the theophylline release when the pectin HM content of the coating ranged between 10.0 and 15.0% /w (related to the Eudragit RL).

Ishibashi *et al.*, (1998) scintigraphic study was to provide "proof of concept" for a novel capsule-type colonic delivery system (Colon-Targeted Delivery Capsule) in healthy volunteers. The human data validates the design concept behind the release mechanism, in that capsule disintegration, and hence drug release, did not start until 5 h after gastric emptying, irrespective of whether the product was administered to fasted or fed subjects. However, the potential for prolonged gastric residence for large enteric coated products intended for intestinal targeting was also observed; overall, the study provides a focus for subsequent product development and highlights the role of scintigraphy in dynamically visualizing the drug delivery process.

DRUG PROFILE

CAPECITABINE

Capecitabine is a new anticancer therapy for oral administration. It is a fluoro pyrimidine which is converted into 5-fluoro uracil once inside the tumour. The drug takes advantage of the higher thymidine phosphorylase activity in malignant tissue. Its activity and safety had already been tested in two large phase –III trials, where it provided to be superior to 5-fluoro uracil / leucovorin

Molecular formula	–	$C_{15}H_{22}FN_3O_6$
Molecular weight	–	359.35
Melting point	–	110 - 121°C

Chemical Name

1 – (5Deoxy β – D – ribofuranosyl – 5 - fluoro – 1,2 – dihydro – 2 – oxo – 4 – pyrimidimyl) – Carbamic acid pentyl ester.

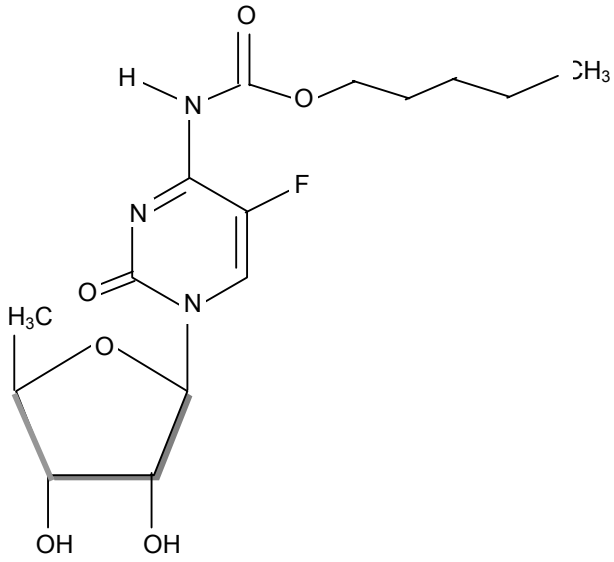
Solubility

Soluble in dichloromethane and methanol, Slightly soluble in water.

Composition

C	-	50.14%
H	-	6.17%
F	-	5.29%
N	-	11.69%
O	-	26.71%

Chemical Structure



BIO ACTIVATION

Capecitabine is readily absorbed from the gastro intestinal tract. In the liver, a 60 K Da Carboxylestesterase hydrolyzes much of the 6 compound to 5 – deoxy – 5- fluorocyticline [5-DFCR] Cytidine deaminase, an enzyme found in most tissues, including tumors, subsequently converts 5-DFLT to 5- deoxy – 5- fluorouridine [5-DFUR] . The enzyme thymidine phosphorylase then hydrolyzes 5 – DFUR to the active drug 5 fluoro uracil. Human carcinomas express these enzymes in higher concentrations than surrounding normal tissues.

Both normal & tumor cell metabolize 5-Fu to 5-fluoro-2-deoxyyuridine mono pho 5phate & 5-flurousidine triphosphate these metabolites cause cell injury by two different mechanisms. First 5 – fluoro – 2 - deoxyuridine monophosphate & the folate cofactor N^{5-10} – methylenetetrahydrofolate find to thymidylate synthase (T5) to form a covalently bound ternary complex. This finding inhibits the formation of thymidylate from 2-deoxyuridylate Thymidylate is the necessary precursor of thymidine triphosphate which is essential for the synthesis of DNA, so that a deficiency of this compound can inhibit cell division second, nuclear transcriptional enzymes can mistakenly incorporate FUTP in place of uridine triphosphate during the synthesis of RNA. This metabolic error can interfere with RNA processing & protein synthesis.

PHARMACOKINETICS

Capecitabine is rapidly & almost completely absorbed unchanged from the gastrointestinal tract, food decrease rate and extent of absorption but the clinical significance is unclear. Capecitabine is recommended to be taken with food because its efficacy & safety are based on studies when it was given within 30 min after a meal. It takes 1.5 hours to attain peak plasma concentration 60% of the drug is bound to plasma proteins. It is metabolized in the liver to 5 – deoxy-5- fluorocytidine [5-DFCR] and then to 5-deoxy -5 fluorouridine [5-DFUR] in liver and tumour tissues. Where it interfere in the synthesis of DNA & RNA processing & protein synthesis. It is mainly excreted through urine (96%).

USES

Apart from colorectal carcinoma it is also used in breast cancer and pancreatic cancer.

DOSAGE

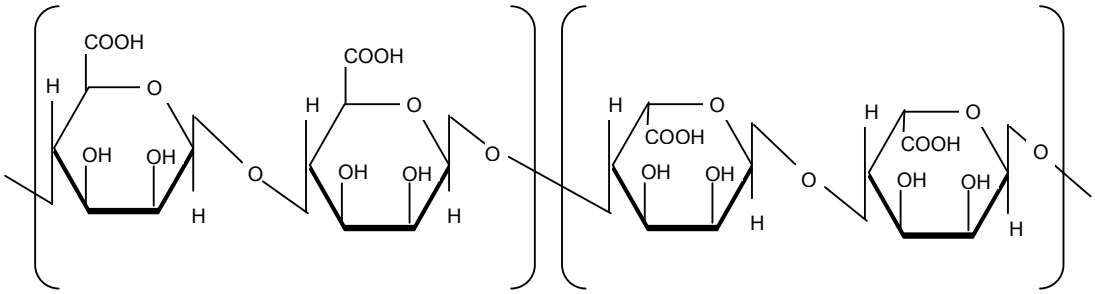
Capecitabine is administrated orally – 1250mg/m^2 [range 313 – 1250 mg/m^2] twice a day for 14 consecutive days starting on day 1 [total dose per cycle 35000 mg/^{2} [range 8764-35000mg/m^2] followed by a 1 week rest period given as 3 week cycles.

ADVERSE EFFECT

The most common adverse effect of capecitabine are diarrhea, nausea, vomiting, abdominal pain, Stomatitis, anemia, Lymphopenia, dermatitis, bronchospasm fatigue, hyperbilirubinemia, pyrexia, dehydration. (AAHFS Drug introduction *et al.,* 2004; Subramanian *et al.,* 2002).

POLYMER PROFILE

Sodium alginate



Sodium alginate is water soluble salt of alginic acid which is a polysaccharide extracted from marine brown algae. It contains two uronic acids β- D – Mannuronic acid (M) and α – L – glucouronic acid (G) and it is composed of homopolymeric block MM or GG and blocks with an alternating sequence (MG block). Many grades of sodium alginate are available and are selected depending upon the purpose, e.g. high L – glucouronic acid content gives high gel strength. Most of the alginic acid consists of the homopolymeric block of D- Mannuronic acid and L- glucouronic acid.

Sodium Alginate is a white or slightly yellow powder freely soluble in hot or cold water, a four percent (4%) solution giving a thick viscous fluid. The solution is not coagulated by heat and does not set to jelly on

cooling. It can be made to gel by converting it partly into a calcium salt by adding a

small proportion of calcium chloride alginate microsphere are prepared by suspending the drug or protein in sodium alginate solution and spraying this solution into 1.3% W/U buffered calcium chloride to form cross linked micro capsules.

Another method is modified emulsion method in this the drug is suspended in sodium alginate solution and add this suspension into primary emulsion (liquid paraffin heavy with span 80) drop wise to form W/o type of emulsion to this add calcium chloride drop wise form cross linked microspheres.

Function

Used as stabilizing agent, suspending agent, tablet and capsule disintegrant, tablet binder, viscosity increasing agent.

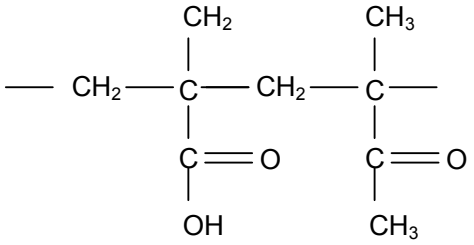
Application in pharmaceutical formulation

Sodium alginate is used in variety of oral and topical pharmaceutical formulation.

EUDRAGIT S 100

Eudragit S 100 is a anionic copolymers based on methacrylic acid and methyl methacrylate. The ratio of the free carboxyl groups to the ester groups is approximately. 1:2 in eudragit S -100. The average molecular weight is 135,000.

Structure



Description

White powders with a faint characteristic odour

Solubility

1 g of Eudragit s -100 is dissolves in 7 g methanol, ethanol, in aqueous isopropyl alcohol and acetone (containing approx. 3 % water), as well as in 1 N sodium hydroxide to give clear to slightly cloudy solutions. Insoluble in ethyl acetate, methylene chloride, petroleum ether and water.

Identity testing

Assay

The assay is performed according to" Potentiometric titration" approximately. 0.5 g eudragit S-100 are dissolved in 60 ml isopropyl

alcohol and 40 ml water with stirring for 30 to 60 minutes at 50 °C.

Sodium hydroxide (NaOH) 0.5 N is used as the titrant. Under the same conditions, a blank value is determined. 1 ml 0.5 N NaOH corresponds to 43.045 mg methacrylic acid units.

Viscosity / Apparent viscosity

Eudragit S-100: 50 - 200 mPa. The viscosity of the Test solution is determined by means of a Brookfield viscometer (spindle 1 /30 rpm / 20 °C).

Refractive index

1.390 - 1.395.

Relative density

0.831 - 0.852.

Purity

Sulphated ash / Residue on ignition

Max. 0.1 %

Heavy metals

Max. 20 ppm

Arsenic

Max. 2 ppm

Storage

Protect from warm temperatures protect against moisture(Raymondetal.,1992;Internet:WWW.roehm.com).

METHODOLOGY

MATERIALS USED

Pure Drug

1.	CAPECITABINE	-	Dr Reddy`s Labs, Medak Dist:, A.P., India (gift sample)

Polymer & Excipient

2.	Sodium Alginate	-	Loba Chemie Pvt Ltd Mumbai
3.	Eudragit S 100	-	Rohm pharma .,Germany (gift sample)
4.	Liquid paraffin heavy	-	Loba Chemie Pvt Ltd Mumbai
5.	Tween 80	-	Loba Chemie Pvt Ltd Mumbai
6.	Span 80	-	Loba Chemie Pvt Ltd Mumbai
7.	Petroleum ether	-	Qualigens fine chemicals Mmbai
8.	Methanol	-	Loba Chemie Pvt Ltd Mumbai
9.	Dichloromethane	-	Loba Chemie Pvt Ltd Mumbai
10.	Dibutyl phthalate	-	Loba Chemie Pvt Ltd Mumbai
11.	Distilled water	-	Ultra pure Pvt Ltd Coimbatore

Chapter VI
Methodology

INSTRUMENTS USED

1.	Mechanical stirrer	-	Universal motors Mumbai
2.	Vacuum dessicator		
3.	Magnetic stirrer 2MLH	-	REMI Instruments, Vasai.
4.	Dissolution rate test apparatus USP XXII	-	Dolphin, Mumbai
5.	pH meter LI-120	-	Elico, Mumbai
6.	Electronic weighing balance ELB-300	-	Shimadzu, Japan.
7.	High Sensitive digital Balance HR 200	-	AND Japan
7.	Scanning Electron Microscope-6400		Jeol Jsm , Japan
8.	UV- Visible Spectrophotometer UV-1650PC	-	SHIMADZU, Japan.
9.	Infrared Spectrophotometer FTIR-8400S	-	SHIMADZU, Japan
10.	KBr PRESS model M-15	-	Techno search instruments Chennai

Chapter VI
Methodology

PREFORMULATION STUDIES

Infrared (IR) absorption spectroscope

To investigate any possible interaction between the drug and the polymer, the IR spectra of pure drug capecitabine, its physical mixture sodium alginate were carried out using Infrared spectrophotometer FTIR-8400S Shimadzu (TOKY0 JAPAN). The sample were prepared as KBr disks compressed under a pressure 8 ton/nm^2 and the wave length selected ranged between 400-4000cm^{-1}.

The IR spectrum of the physical mixture was compared with those of pure drug and polymer to detect any appearance or disappearance of peaks.

Fig.3 IR SPECTRUM OF CAPECITABINE

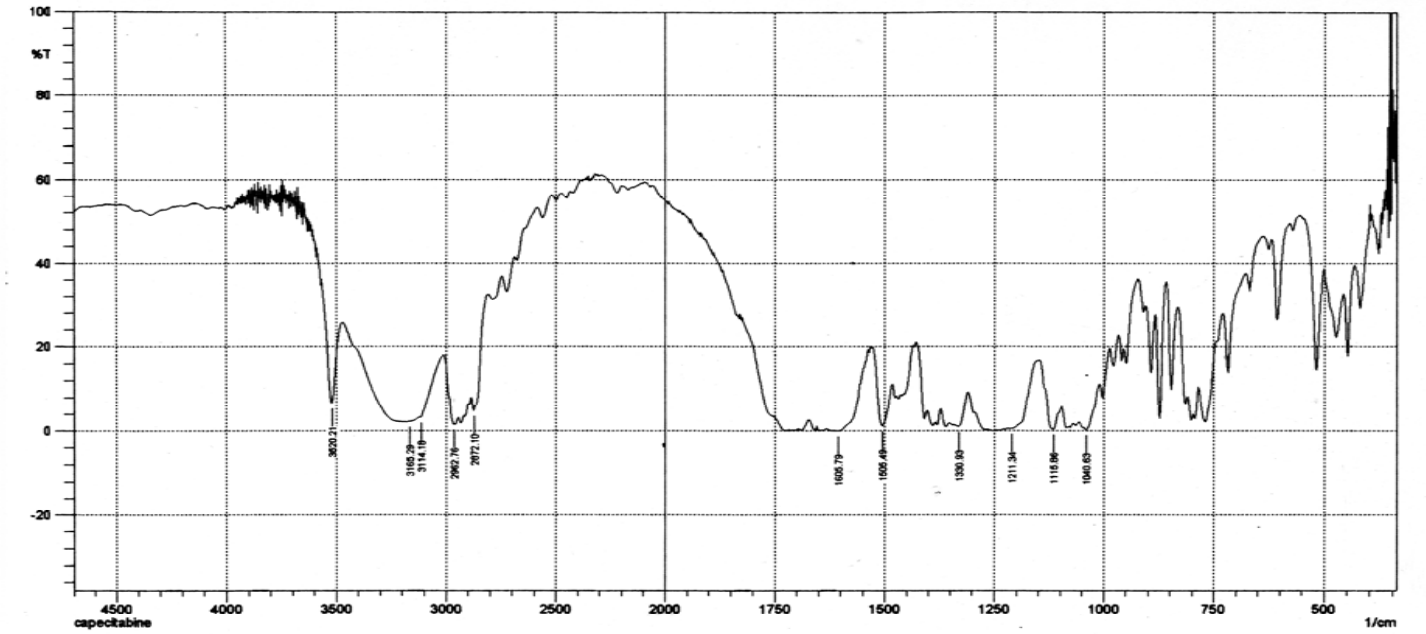

100
%T
80
60
40
20
0
-20
4500
4000
3500
3000
2500
2000
1750
1500
1250
1000
750
500
1/cm
capecitabine
3420.21
3165.29
3114.19
2957.76
2872.10
1608.79
1505.49
1330.99
1211.34
1118.86
1046.63
Comment;
capecitabine
No. of Scans; 10
Resolution; 2 [1/cm]
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Fig. 4 IR SPECTRUM OF SODIUM ALGINATE

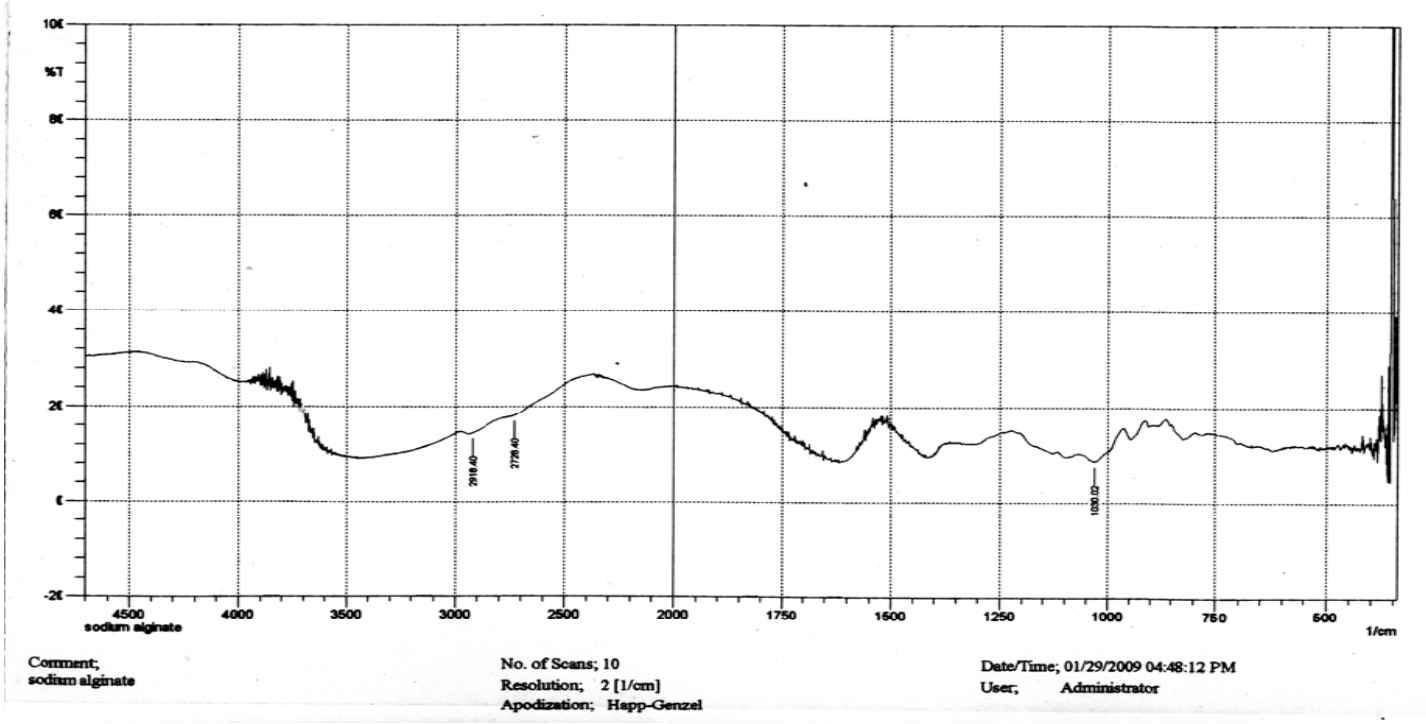
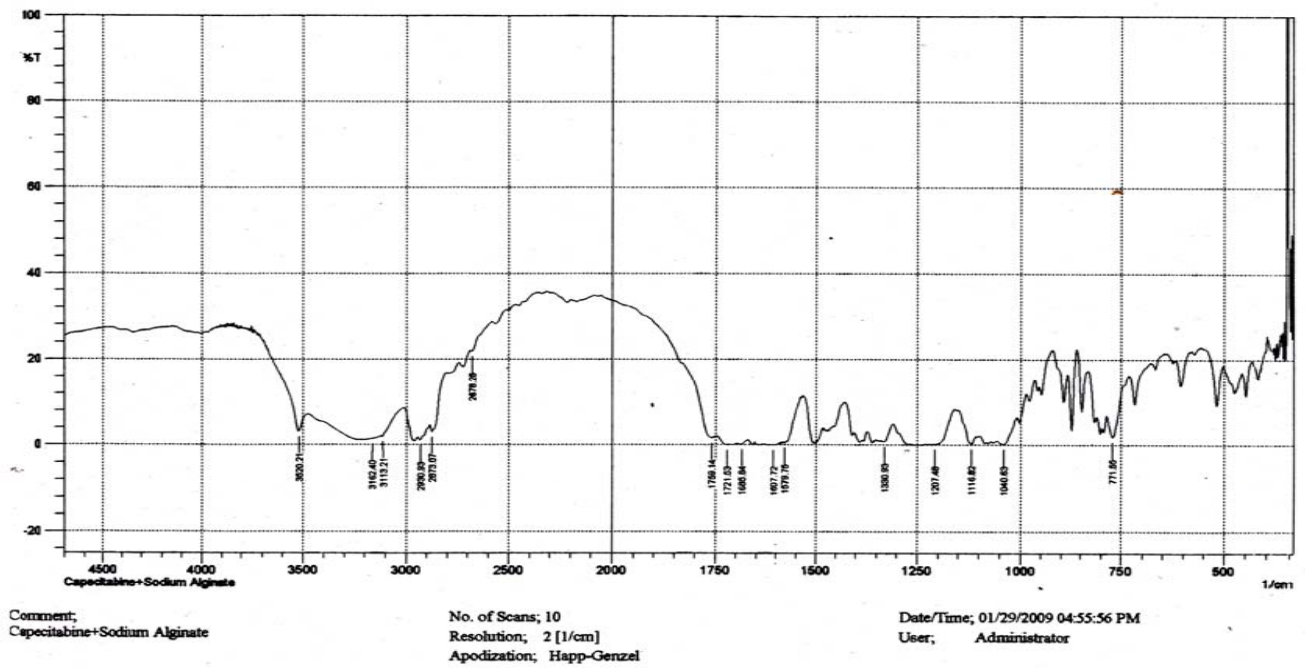


Fig.5 IR SPECTRUM OF PHYSICAL MIXTURE



Chapter VI
Methodology

PREPARATION OF STANDARD GRAPH OF CAPECITABINE

An accurately weighed quantity of 100mg Capecitabine was transferred into a 100ml standard flask and volume was made up to the mark using Phosphate buffer of pH 7.6.

From the primary stock solution 2ml was transferred to 100 ml volumetric flask and diluted upto the mark using Phosphate buffer of pH 7.6.The concentration of the solution will be 20μg/ml. Various concentrations 2μg,4μg, 6μg, 8μg, 10μg, 12μg, 14μg, 16μg, 18μg and 20μg were prepared by diluting 1ml, 2ml, 3ml, 4ml, 5ml, 6ml, 7ml, 8ml, 9ml and 10ml of the stock solution to 10ml using buffer of pH 7.6 respectively. The absorbance was noted at 239nm using UV/Vis Spectrophotometer.

Similarly the various standard graphs of capecitabine were prepared in 0.1 N HCl pH 1.2 and with phosphate buffer of pH 6.8..

Table 1 : Standard graph of capecitabine at pH 7.6

Concentrations (μg/ml)	Absorbance (239nm)
2	0.07886
4	0.14136
6	0.23059
8	0.31946
10	0.37915
12	0.45410
14	0.53394
16	0.61365
18	0.68933
20	0.76611

Fig.6 : Standard graph of capecitabine at pH 7.6

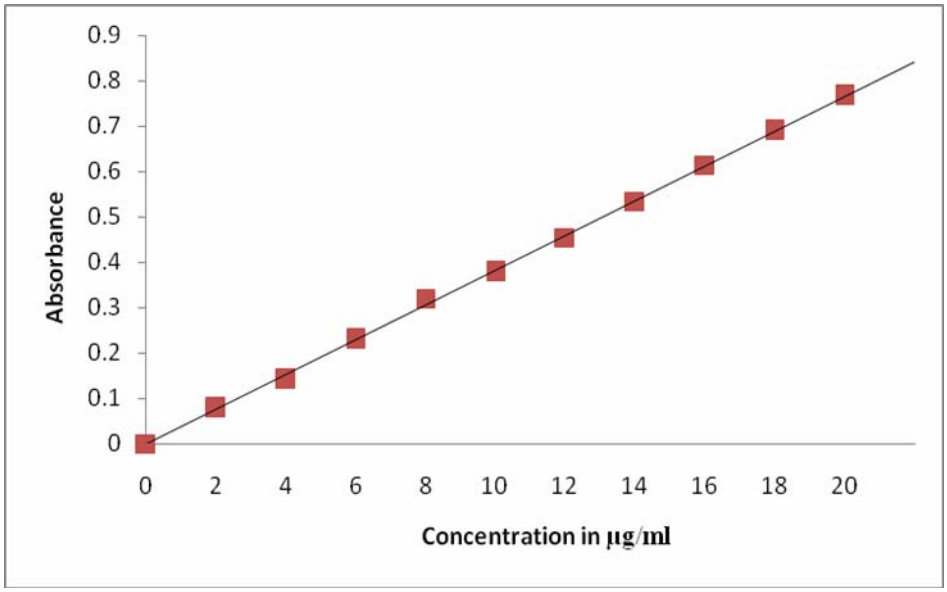


Table 2 : Standard graph of capecitabine at pH 6.8

Concentrations (μg/ml)	Absorbance (239nm)
2	0.07918
4	0.16136
6	0.25595
8	0.34093
10	0.44078
12	0.54246
14	0.63000
16	0.71958
18	0.82859
20	0.91366

Fig.7: Standard graph of capecitabine at pH 6.8

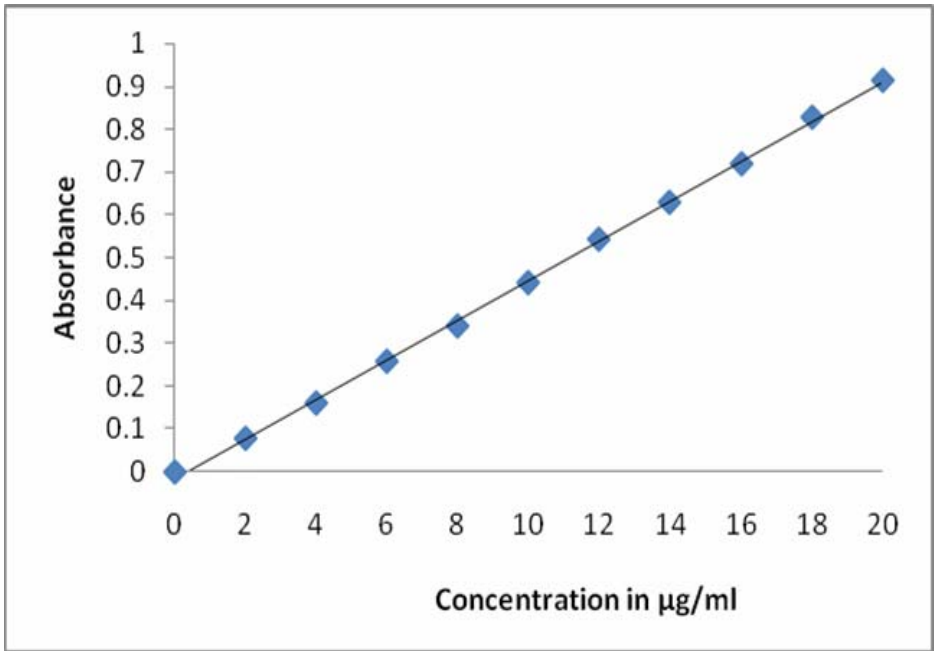
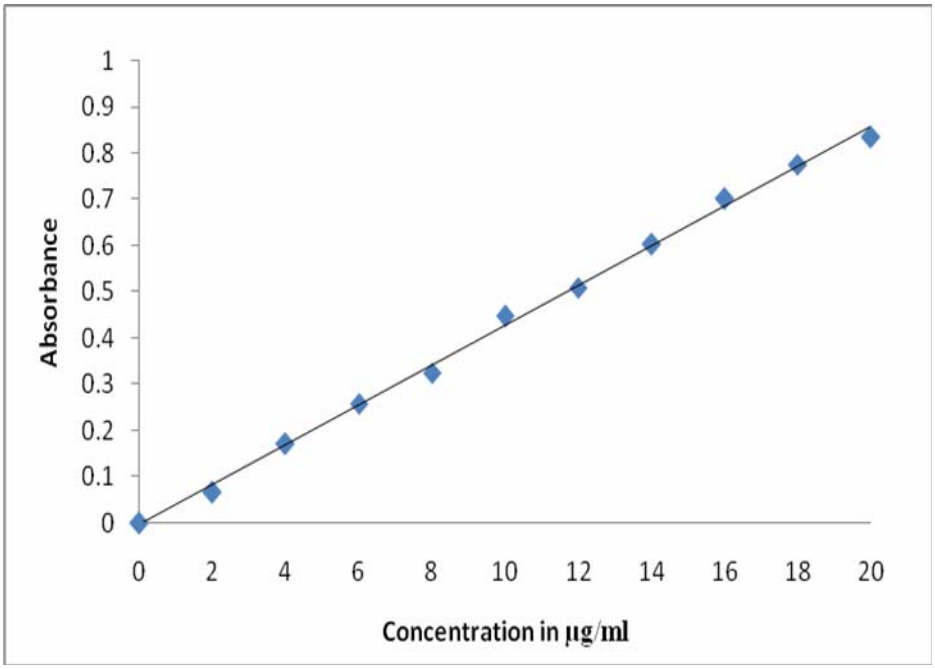


Table 3: Standard graph of capecitabine at pH 1.2

Concentrations (µg/ml)	Absorbance (239nm)
2	0.06763
4	0.17236
6	0.25879
8	0.32554
10	0.44726
12	0.51013
14	0.60339
16	0.70144
18	0.77563
20	0.83484

Fig. 8 : Standard graph of capecitabine at pH 1.2



Chapter VI
Methodology

PREPARATION OF SODIUM ALGINATE MICROSPHERES CONTAINING CAPECITABINE

Sodium alginate microspheres containing capecitabine were prepared by using single emulsion technique. Heavy liquid paraffin was used as the external phase and aqueous solution of Sodium alginate was used as dispersed phase.

Procedure

Drug and polymer were weighed as per the table (Table -2) and mixed well to make a homogeneous drug polymer solution.

The 10ml of the drug polymer solution is added drop wise into 30ml of heavy liquid paraffin containing 2 to 4 drop (0.2%) span 80 and stirred for 90 minutes. Different concentrations of calcium chloride 6ml as per the table was added to the emulsion and stirred for 15 minutes. The prepared microspheres were washed with petroleum ether for several times and filtered and dried (ziyaur et al., 2006;Asha et al.,2006).

Preparation of eudragit –S- 100 coated gelatin capsule

Dipping method is used for coating the microspheres containing hard gelatin capsule. Hard gelatin capsule (size No 1) was taken, cap and body is separated. Each part is dipped into 10% w/v Eudragit S- 100 solution in the methanol and dichloro methane mixture (1:4) along with 2% v/v dibutyl phthalate as plasticizer, for 6 times with an interval of 3hrs. The coated body is filled with accurate amount of microspheres and sealed (Ishibashi et al.,1998;Jain et al.,2005;Ziyaur et al.,2006)

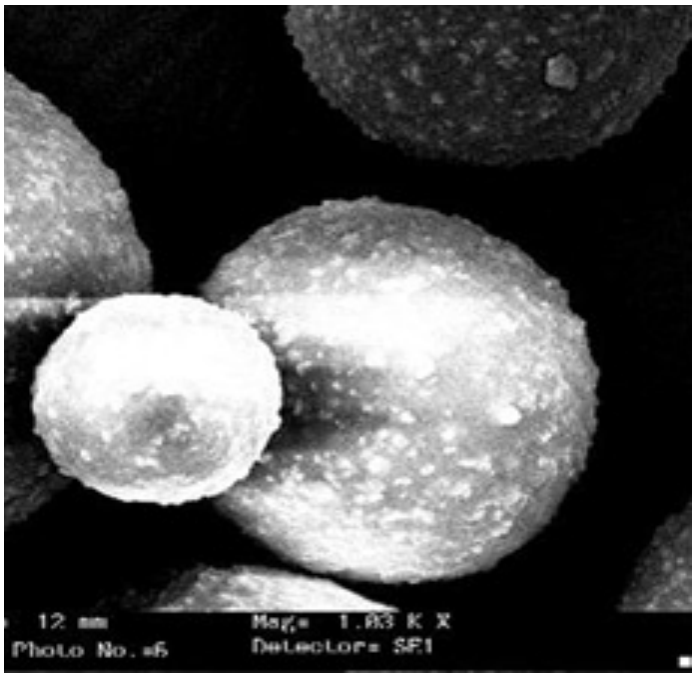
Table 4 : Various formulation of capecitabine containing alginate microspheres

Sl No.	Formulation Code	Drug used in Formulation (mgs)	Volume of Polymer used in Formulation (ml)	Drug : Polymer Ratio	Percentage of cross linking agent
1	FA1	125	3.125 (4%)	1:1	2 (6ml)
2	FA2	125	6.25 (4%)	1:2	2 (6ml)
3	FA3	125	3.125 (4%)	1:1	5 (6ml)
4	FA4	125	6.25 (4%)	1:2	5 (6ml)
5	FB1	125	2.5 (5%)	1:1	2 (6ml)
6	FB2	125	5 (5%)	1:2	2 (6ml)
7	FB3	125	2.5 (5%)	1:1	5 (6ml)
8	FB4	125	5 (5%)	1:2	5 (6ml)
9	FC1	125	2.08 (6%)	1:1	2 (6ml)
10	FC2	125	4.17 (6%)	1:2	2 (6ml)
11	FC3	125	2.08 (6%)	1:1	5 (6ml)
12	FC4	125	4.17 (6%)	1:2	5 (6ml)

DETERMINATION OF SIZE AND SHAPE OF MICRO PARTICLES BY SCANNING ELECTRON MICROSCOPE

The surface morphology and internal texture of Capecitabine micro particles were observed by a scanning electron microscope. Scanning electron microscope photographs were taken on JSM 6400 Scanning electron microscope at 3K magnification at room temperature. Before scanning the micro particles were sputtered with gold to make the surface conductive. The Fig. 9 shows that the particles are spherical enough and having rough surface.

Fig. 9 SEM photograph



DETERMINATION OF PARTICLE SIZE OF MICROPARTICLES BY OPTICAL MICROSCOPIC METHOD

Clean the microscope and focus for bright light. Eye piece micro meter is fixed and calibrated using stage micrometer. Few drops of samples is mounted on the clean glass slide minimum of 200 particle is counted and the mean size is calculated using the formula.

Arithmetic Mean = $\Sigma nd / \Sigma n$

Table 5 : Particle size distribution of formulations FA1,FA2,FA3,FA4

FORMULATION CODE	PARTICLE SIZE RANGE(μM)	MEAN DIAMETER (μm)(d)	NUMBER OF PARTICLES(n)	nxd
FA1	0 – 10	5	43	215
	10 – 20	15	108	1620
	20 – 30	25	32	800
	30 – 40	35	17	595
FA2	0 – 10	5	53	265
	10 – 20	15	107	1605
	20 – 30	25	30	750
	30 – 40	35	10	350
FA3	0 – 10	5	32	160
	10 – 20	15	108	1620
	20 – 30	25	40	1000
	30 – 40	35	20	700
FA4	0 – 10	5	61	305
	10 – 20	15	79	1185
	20 – 30	25	43	1075
	30 – 40	35	17	595

The Arithmetic Mean of particle size

FA_1 = 16.15μm, FA_2 = 14.86 μm, FA_3 = 17.4 μm, FA_4 = 15.8 μm

Fig. 10 Particle size distribution of formulations FA1,FA2,FA3,FA4

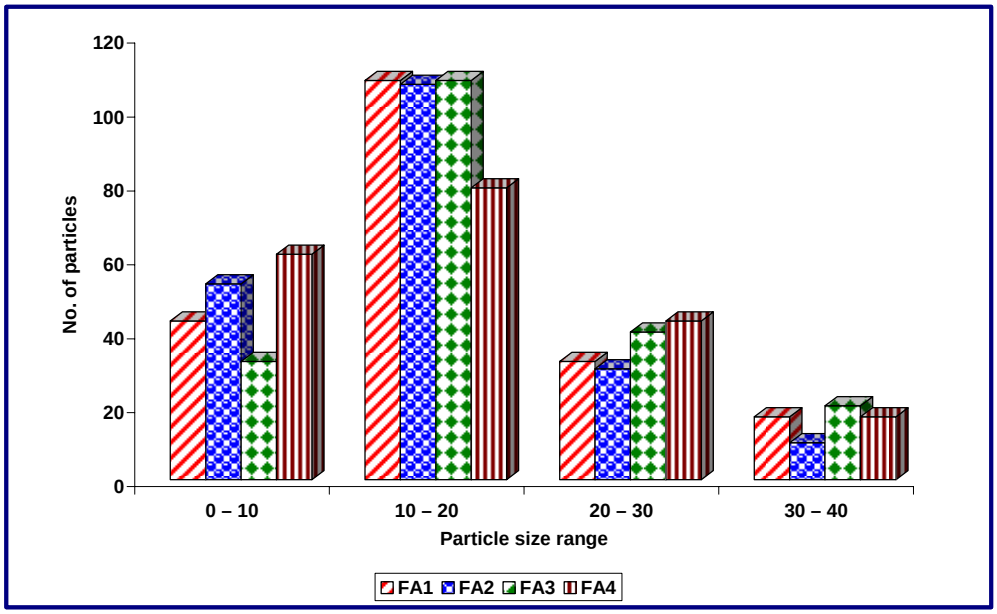


Table 6: Particle size distribution of formulations FB1, FB2, FB3, FB4

FORMULATION CODE	PARTICLE SIZE RANGE (μM)	MEAN DIAMETER (μm)(d)	NUMBER OF PARTICLES (n)	nxd
FB1	0 – 10	5	57	285
	10 – 20	15	83	1245
	20 – 30	25	32	800
	30 – 40	35	28	980
FB2	0 – 10	5	49	245
	10 – 20	15	83	1245
	20 – 30	25	30	750
	30 – 40	35	38	1330
FB3	0 – 10	5	23	115
	10 – 20	15	76	1140
	20 – 30	25	47	1175
	30 – 40	35	54	1890
FB4	0 – 10	5	17	85
	10 – 20	15	73	1095
	20 – 30	25	50	1250
	30 – 40	35	60	2100

The Arithmetic Mean of particle size

FB_1 = 16.55 μm, FB_2 = 14.86 μm, FB_3 = 21.6μm, FB_4 = 22.65

Fig. 11 : Particle size distribution of formulations FB1, FB2, FB3, FB4

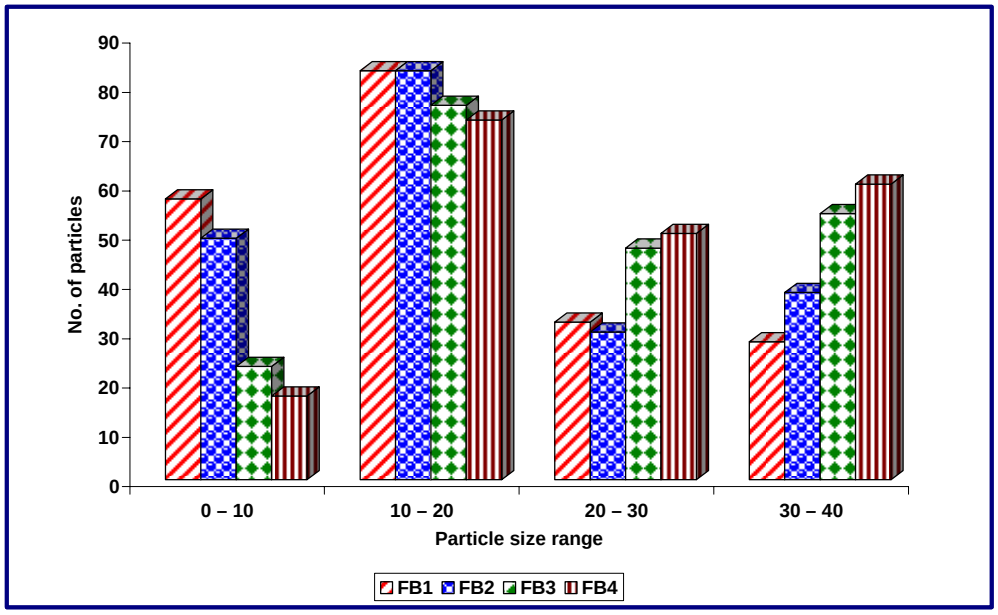


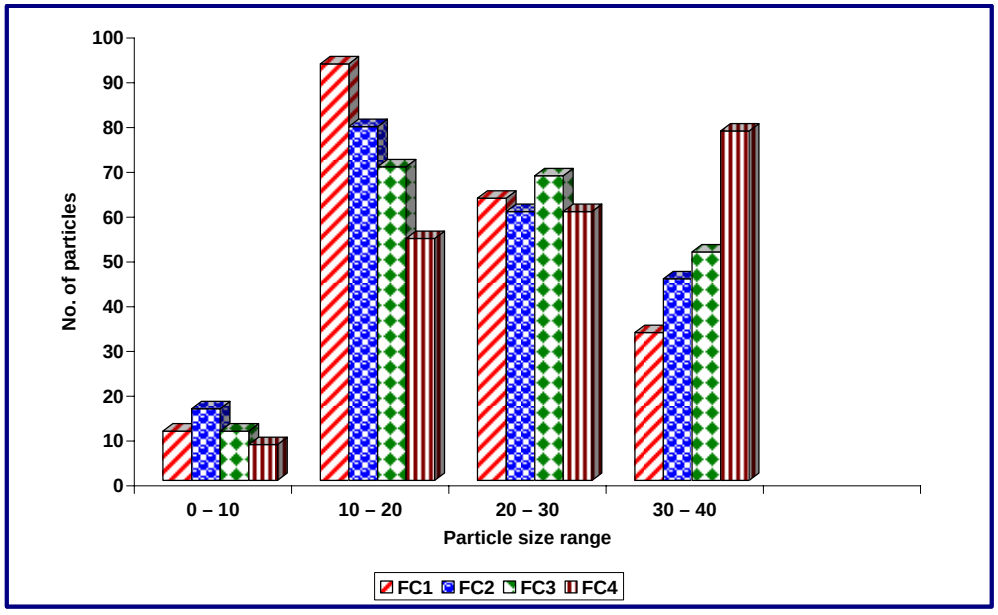
Table 7: Particle size distribution of formulations FC1,FC2, FC3, FC4

FORMULATION CODE	PARTICLE SIZE RANGE (μM)	MEAN DIAMETER (μm)(d)	NUMBER OF PARTICLES (n)	nxd
FC1	0 – 10	5	11	55
	10 – 20	15	93	1395
	20 – 30	25	63	1575
	30 – 40	35	33	1155
FC2	0 – 10	5	16	80
	10 – 20	15	79	1185
	20 – 30	25	60	1500
	30 – 40	35	45	1575
FC3	0 – 10	5	11	55
	10 – 20	15	70	1050
	20 – 30	25	68	1700
	30 – 40	35	51	1785
FC4	0 – 10	5	8	40
	10 – 20	15	54	810
	20 – 30	25	60	1500
	30 – 40	35	78	2730

The Arithmetic Mean of particle size

FC_1 = 20.9 μm, FC_2 = 21.7 μm, FC_3 = 22.95 μm, FC_4 = 25.4 μm

Fig. 12 : Particle size distribution of formulations FC1,FC2, FC3, FC4



ENTRAPMENT EFFICIENCY

25mg of drug loaded core microspheres was weighed and washed with 10ml of phosphate buffer of pH 6.8 to remove the surface – associated drug. Then microspheres were kept in phosphate buffer of pH 7.6 for digestion for 24hrs and sonicated for 1 hr at room temperature. From that 1ml of sample is withdrawn and diluted 1000 times using phosphate buffer pH 7.6 and quantified spectrophotometrically at 239nm (Ziyaur et al.,2006).

Entrapment efficiency is determined by using the formula.

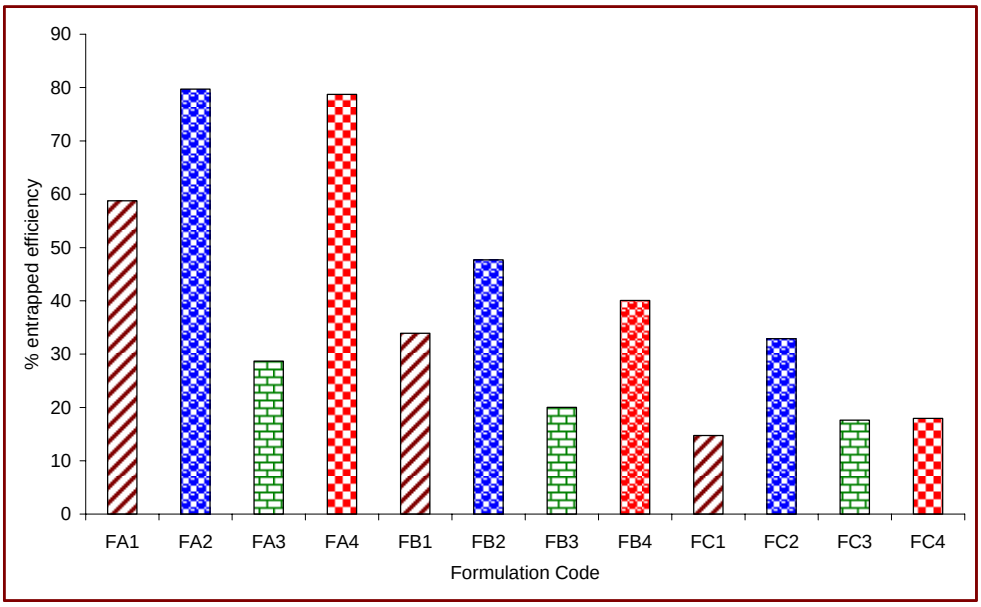
Practical yield from graph

$$\% \text{ entrapment} = \frac{\text{Partical yield from graph}}{\text{Initial amount loaded}} X\ 100$$

Table 8 : Percentage entrapment efficiency of formulations

S.No.	Formulation Code	Practical Yield in mg	Absorbance in nm	Graph yield in μgm	Percentage entrapped efficiency
1	FA_1	170	0.412	10.8	58.75±0.89
2	FA_2	228.5	0.417	10.9	79.7±0.75
3	FA_3	299	0.113	3	28.7±0.52
4	FA_4	253.6	0.376	9.7	78.73±1.09
5	FB_1	230	0.175	4.6	33.92±0.23
6	FB_2	216	0.262	6.9	47.7±0.57
7	FB_3	179	0.135	3.5	20.05±0.42
8	FB_4	167	0.286	7.5	40.06±0.41
9	FC_1	271	0.062	1.7	14.74±0.24
10	FC_2	367.1	0.107	2.8	32.9±o.31
11	FC_3	367.2	0.056	1.5	17.62±o.82
12	FC_4	295.2	0.072	1.9	17.95±0.13

Fig. 13 Percentage entrapment efficiency of formulations



IN VITRO RELEASE STUDIES

In vitro drug release was studied using temperature controlled magnetic stirrer, with 10ml of dissolution medium maintained at 37°C at 60 rpm.

Procedure

5mg of core microspheres were suspended in 10ml of phosphate buffer (pH 7.6) containing 0.02% w/v tween 80. 1ml of sample were withdrawn at specified time intervals 15min, 30min, 45min, 60min, 75min, 90min, 105min, and 120min replaced with 1ml fresh phosphate buffer pH 7.6. Dissolution sample is further diluted to 100 times using phosphate buffer pH 7.6 solution and quantified spectrophotometrically at 239nm(Ziyaur et al.,2006).

Table 9: *In vitro* dissolution profile of 4% alginate microspheres with different drug polymer ratio and % of cross linking agent formulations FA1,FA2,FA3,FA4

Time in minute	Percentage of release			
	FAI	FA2	FA3	FA4
15	3.54±0.26	44.61±0.11	38.10±0.18	27.54±0.25
30	37.25±0.18	50.13±0.19	49.14±0.09	47.89±0.21
45	38.9±0.41	57.77±0.43	58.51±0.20	51.25±0.12
60	47.88±0.36	58.50±0.29	67.77±0.31	67.11±0.23
75	49.78±0.21	64.35±0.31	78.12±0.73	69.39±0.15
90	50.05±0.45	67.77±0.52	84.13±0.63	78.55±0.12
105	54.01±0.75	86.29±0.34	87±1.03	79.81±0.19
120	54.41±0.32	87.11±0.78	92.02±1.23	81.91±0.01

Fig.14: *In vitro* dissolution profile of 4% alginate microspheres with different drug polymer ratio and % of cross linking agent formulations FA1,FA2,FA3,FA4

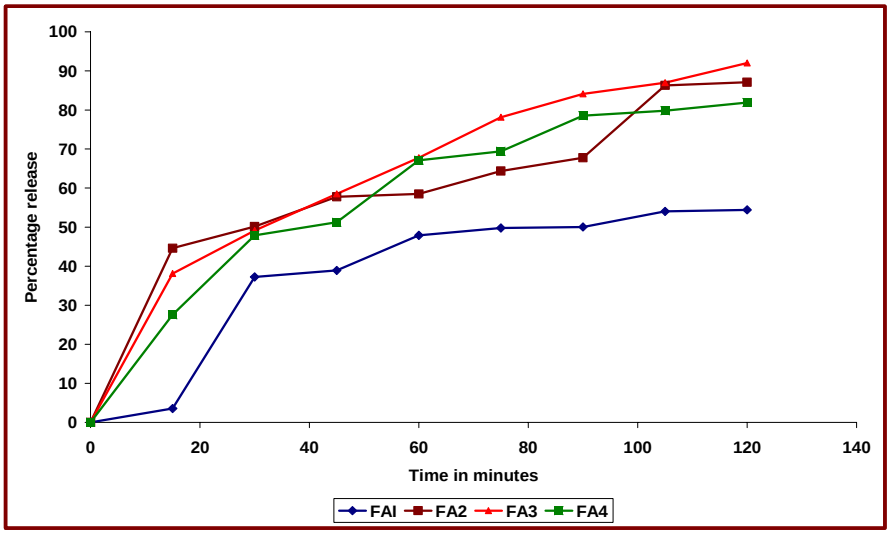


Table 10: *In vitro* dissolution profile of 5% alginate microspheres with different drug polymer ratio and % of cross linking agent formulations FB1,FB2,FB3,FB4

Time in minute	Percentage of release			
	FBI	FB2	FB3	FB4
15	15.62± 0.27	48.58±0.18	35.18±0.23	37.05±0.21
30	18.81±0.22	66.98±0.19	36.33±0.18	37.43±0.22
45	20.54±-0.19	68.19±0.28	39.18±0.19	47.16±0.18
60	24.88±0.20	69.93±0.13	46.34±0.20	48.28±0.16
75	27.78±1.19	78.76±0.18	52.06±0.12	52.4±0.18
90	30.09±1.20	80.97±0.17	55.21±0.13	52.8±0.16
105	32.12±0.22	85.39±0.19	60.64±0.19	52.52±0.20
120	36.46±0.16	86.12±0.14	61.24±0.20	68.11±0.29

Fig.15: *In vitro* dissolution profile of 5% alginate microspheres with different drug polymer ratio and % of cross linking agent formulations FB1, FB2, FB3,FB4

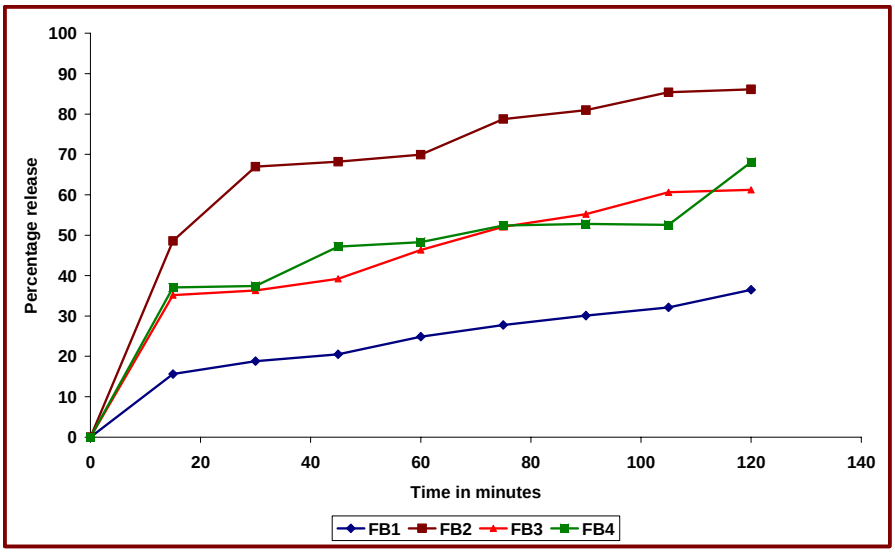


Table 11: *In vitro* dissolution profile of 5% alginate microspheres with different drug polymer ratio and % of cross linking agent formulations FC1, FC2, FC3, FC4

Time in minute	Percentage of release			
	FCI	FC2	FC3	FC4
15	7.36±0.18	14.67±0.11	25.24±0.18	14.16±0.18
30	12.99±0.13	23.48±0.12	29.94±0.17	16.52±0.17
45	28.58±0.13	31.12±0.18	35.81±0.16	21.71±0.15
60	32.48±0.11	41.10±0.19	47.56±0.15	31.16±0.21
75	54.57±0.19	48.15±0.14	58.72±0.21	39.8±0.22
90	59.29±0.20	59.30±0.14	65.77±0.22	40.60±0.12
105	60.63±0.18	61.1± 0.13	81.03±0.21	.41.1±0.12
120	72.33±0.20	69.28±0.18	82.79±0.23	43.32±0.12

Fig.16 : *In vitro* dissolution profile of 5% alginate microspheres with different drug polymer ratio and % of cross linking agent

formulations FC1, FC2, FC3, FC4

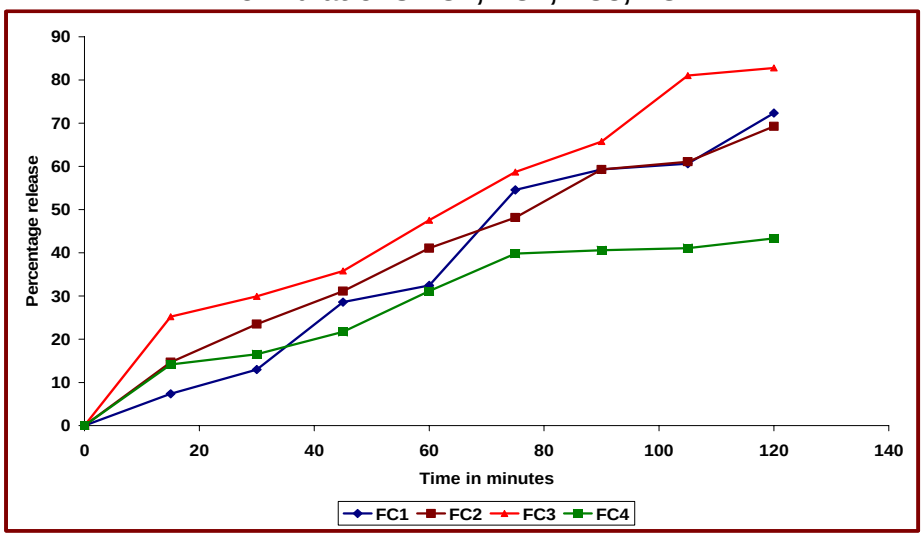
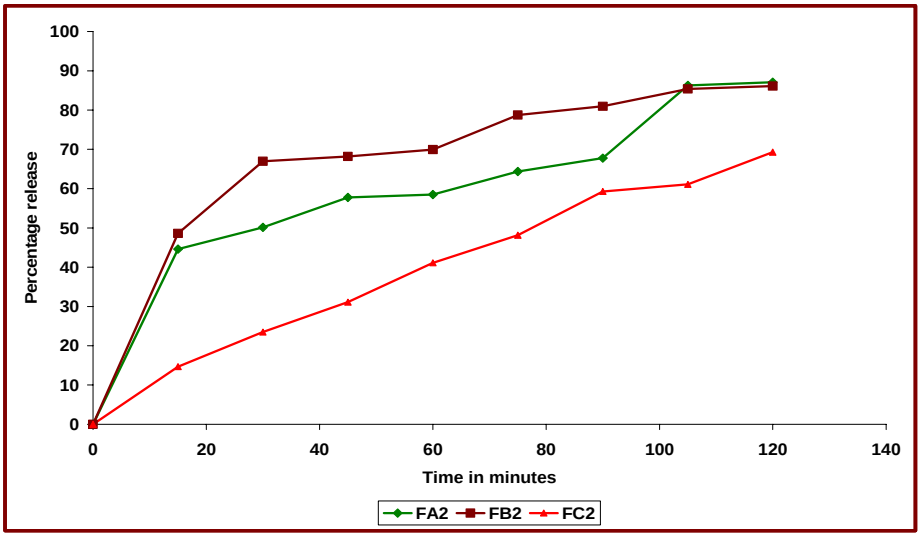


Table 12 : Comparative *In vitro* dissolution profile of best formulations of 4%,5%,6% alginate microspheres FA2, FB2, FC2

Time in minute	Percentage of release		
	FA2	FB2	FC2
15	44.61±0.11	48.58±0.18	14.67±0.11
30	50.13±0.19	66.98±0.19	23.48±0.12
45	57.77±0.43	68.19±0.28	31.12±0.18
60	58.50±0.29	69.93±0.13	41.10±0.19
75	64.35±0.31	78.76±0.18	48.15±0.14
90	67.77±0.52	80.97±0.17	59.30±0.14
105	86.29±0.34	85.39±0.19	61.1± 0.13
120	87.11±0.78	86.12±0.14	69.28±0.18

Fig. 17 Comparative *In vitro* dissolution profile of best formulations of 4%, 5%, 6% alginate microspheres FA2, FB2, FC2



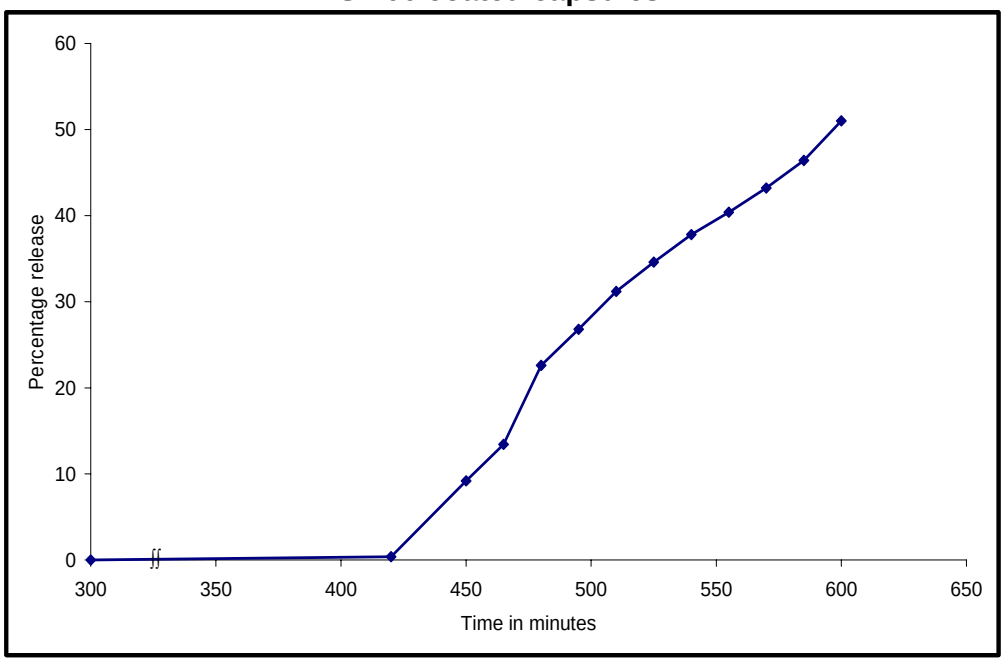
***Invitro* release studies from coated capsule**

Microspheres equivalent to 50mg of capecitabine were filled in a capsule (size No1) and sealed. Dissolution studies carried out in 900ml of pH progression medium at 37^0C and 60 rpm. The pH of the medium was gradually increased from pH 1.2 (0.1NHCl), pH 5.8, pH 6.8 phosphate buffer for 2 hours each respectively. Then transferred it to pH 7.6 phosphate buffer until the end of the experiment. At specific time interval 1,2,3,4 10hrs dissolution sample is taken diluted to 1000ml using buffer solutions and quantified spectro photometrically at 239nm(Jain et al.,2005; Ziyaur et al.,2006)

Table 13 : *In vitro* dissolution study of formulation (FA2) in Eudragit S-100 coated capsules

Time in minutes	Absorbance In nm	Concentration in mcg/ml	Concentration in mg/900ml	Percentage release
360	0	0	0	0
420	0.016	0.4	0.4	0.39
450	0.175	4.6	4.6	9.2
465	0.296	7.8	7.8	13.44
480	0.432	11.3	11.3	22.6
495	0.511	13.4	13.4	26.8
510	0.591	15.6	15.6	31.2
525	0.662	17.3	17.3	34.6
540	0.719	18.9	8.9	37.8
555	0.771	20.2	20.2	40.4
570	0.826	21.6	21.6	43.2
585	0.85	23.2	23.3	46.4
600	0.935	25.5	25.5	51

Fig.18: *In vitro* dissolution study of formulation (FA2) in Eudragit S-100 coated capsules



RESULTS AND DISCUSSION

COMPATIBILITY STUDIES

The compatibility between the drug Capecitabine and the selected polymer sodium alginate was evaluated using FT-IR peak matching method. The IR spectra of pure drug, polymer and physical mixture (1:1) were shown in spectra Fig: 3, 4, and 5 respectively. There was no appearance or disappearance of peaks in drug and polymer mixture which confirmed the absence of any chemical interaction between drug and polymer.

PARTICLE SIZE AND SHAPE DETERMINATION USING MICROMETER AND SEM

Particle size determination studies were carried out on micrometric properties of the prepared microspheres which are shown in Table 5, 6 and 7. The arithmetic mean size of particles was about 14.86 to 25.4μm

Scanning electron microscopy reveals that the prepared microspheres had a homogeneous solid structure, with no evidences of crystals on the surface Fig. 9. The formulation containing capecitabine 4% sodium alginate 1:2 ratio with 2% calcium chloride (FA2) produced by single emulsion technique enabled us to get spherical, discrete spheres with a size ranging from 10-30μm.

Entrapment efficiency

Entrapment efficiency of alginate microspheres was shown in Table 8. The entrapment efficiency of the microspheres is mainly altered by the drug polymer ratio, percentage of polymer and cross linking agent. The result indicate that the drug polymer 1:2 ratio with 4% polymer and 2% cross linking agent (FA2) shows a highest entrapment efficiency of 79.7%. Similarly 5% and 6% sodium alginate microspheres with the same drug polymer ratio1:2 and 2% percentage of cross linking agent (FB2 and FC2) had good entrapment efficiency 47.7%, 32.9% respectively. The above result clearly indicates higher drug entrapment efficiency at low concentration of polymer.

***IN VITRO* RELEASE STUDIES**.

The *in vitro* release profile of 4% sodium alginate with different drug polymer ratio and percentage of cross linking agent were carried out in phosphate buffer of pH 7.6 and the result is shown in Table 9, 10, 11 and 12 and Fig. 14, 15,16 and 17. *In vitro* release study reveals that drug polymer 1:2 ratio with 2% cross linking agent (FA2) showed a uniform release for 2 hrs than the other formulation containing 4% alginate. So, formulation FA2 was selected as better formulation.

Similar release pattern was observed in 5% and 6% alginate microspheres using 1:2 drug polymer ratio and 2% cross linking agent.

From the three selected formulation of (4% 5% 6% alginate microspheres) 4% alginate microspheres had a good entrapment and release pattern than

the other two formulations.

***IN VITRO* DRUG RELEASE STUDY OF FA4 CONTAINING EUDRAGIT S100 COATED CAPSULES**

Dissolution study was carried out in simulated gastrointestinal tract conditions for Eudragit S100 coated capsule filled with (FA2) microspheres in pH1.2, pH 5.8.and pH 6.8 phosphate buffer revealed that there is no release of capecitabine from 10% Eudragit S100 coated 6 times by dipping method and it shows a good release pattern of capecitabine at pH 7.6 phosphate buffer shown in Table 13 and Fig. 18. The study confirms that release rate of capecitabine from Eudragit S100 coated capsules was found to be pH dependent and is suitable for colon targeting.

SUMMARY AND CONCLUSION

Colorectal carcinoma is the second leading cancer killer and third most common cancer throughout the world. Capecitabine is a new drug for orally administered and widely used for the treatment of colorectal cancer. The first pass metabolism of capecitabine is a problem in achieving bioavailibility. Development of colon targeting microspheres will be the promising technique to avoid first pass metabolism and to achieve the desired bioavailibility profile.

The present investigation is focused on development of capecitabine loaded alginate microspheres and filled in capsules followed by Eudragit-S-100 coating for targeting to colon region. The results suggested that drug, 4% sodium alginate 1:2 ratio with 2% cross linking agent (FA2) was found to be the best formulation and showed 87.1% release in 2hrs with 79.7% entrapment. So the formulation is selected for further coating with Eudragit-S-100 for targeting to colon region. The coated capsules release studies shows no evidence of drug release for up to 6hrs. It is evident that the GI emptying time can be bypassed by the coated capsules the drug release of 51% at the end of 10hrs from coated capsules. Suggests the Eudragit-S-100 coating only degrading in colon pH. The prepared formulation can be further evaluated in vivo for correlating *in vitro* study results. The prepared microspheres is a promising colon targeted delivery device for achieving better kinetic profile with improved bioavailibility.

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